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STUDIES ON THE DIFFERENT PATHWAYS OF EXCHANGE OF MINERALS IN TEETH WITH THE AID OF RADIOACTIVE PHOSPHORUS[1]

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The method of tracing a chemical element in tissues of animals by using a mixture of inactive (normal) and radioactive isotopes of this element was introduced by Hevesy in 1935. In the investigation with the new method, Chievitz and Hevesy (1) studied the phosphorus metabolism in bones and teeth. This study and the following by Hevesy, Holst and Krogh (2), Manly, Le Fevre and Bale (3), Hevesy and Armstrong (4) brought about a series of important results concerning the differences in phosphorus metabolism between growing and adult teeth, between their different parts and different tissues. Teeth of rats, cats and human beings were used in these experiments.

In our investigation we used the above method in combination with establishing certain abnormal metabolic conditions in teeth. By this procedure we investigated the different possible pathways into and within the tooth. As the conditions established in our experiments are partly identical with conditions occurring either spontaneously in pathological altered teeth or by surgical procedure, the results will help us to understand the changes which occur in tooth metabolism as a result of certain pathological processes.

It is clear that there are 3 possible pathways of the metabolism in teeth; namely, through the pulp, the cemento-dentinal junction, and the outer surface of the crown of the tooth. The cemento-dentinal junction is considered here as a boundary of the tooth. With respect to metabolism, the cementum belongs to the periodontal tissues rather than to the tooth itself because of their common blood supply. Even from the anatomical point of view it may be justifiable

[1] Received for publication April 8, 1941.

to consider the cemento-dentinal junction as a boundary of the tooth, as the cementum originates from the periodontal tissues.

The role of the pulp as the main source of nutrition for the dentin is beyond doubt. But very little is known as to the role either the cemento-dentinal junction or the surface of the crown exposed to the saliva may play in the metabolism of the tooth.

The repeatedly stated existence of dentinal tubules which continue into the cementum does not prove that solutions really pass through the cemento-dentinal boundary. There are some statements that in adult teeth, elements like phosphorus are absorbed through the outer surface of the enamel (2, 4). However, nothing is known concerning the pathways of entrance or the possibility of absorption of such substances from the saliva.

MATERIALS AND METHODS

The experimental conditions consisted in either the removal or injury of the pulp and in covering the teeth with a metal cap or crown to protect the enamel from the saliva. By these methods the supply of tissue fluids to the teeth through the pulp or by the saliva could be prevented. For instance, by simultaneously using the cap to cover the enamel and arresting the supply through the pulp, only the cemento-dentinal boundary was left open. To close the latter is impossible, though one might be able to influence the supply through the cemento-dentinal boundary by changing the conditions within the periodontal tissues.

The experiments were performed on the maxillary anterior teeth of 10 adult dogs. The chemical element used was phosphorus. The normal stable phosphorus with the atomic weight 31 was labelled by admixture of the radioactive isotope of the atomic weight 32. For studying the influence of the experimental condition, corresponding incisor teeth on the right and on the left side of the maxilla were compared. For instance, when the pulp of a tooth was removed, the pulp of the corresponding tooth was either left in the normal state or injured. Many combinations of this type were used.

To induce general anesthesia, 5 to 10 cc. of sodium pentothal (thiobarbiturate) were given intravenously. Following complete relaxation, a trachea tube was inserted and ether inhalation instituted.

Following pulp removal an effort was made to hermetically seal the pulp canal with filling material. In some cases in addition to the

routine technique of packing gutta percha cones, a cavity lining consisting of 12 grains of rosin dissolved in 3 fluid drams of chloroform was used to seal the dentinal tubules before placing the gutta percha. To produce an injury to the pulp, the tooth was opened and the pulp punctured until a sign of a slight hemorrhage could be detected. It was immediately capped with eugenol and zinc oxide and the opening sealed with cement. For the crowning of teeth a metal cap was placed so as to prevent the contact of the enamel with the saliva.

The mixture of radioactive and normal phosphorus (which we will call labelled phosphorus) was prepared in the cyclotron of the University of Chicago by bombarding inactive phosphorus with deuterons.[2] Afterwards sodium phosphate was prepared and dissolved in about 10 cc. of distilled water for injection.

The first experiments showed that an activity of 1 millicurie of our labelled phosphorus was necessary to obtain good measurable activities in different parts of the teeth of a dog of about 20 kg. in weight, if the aqueous solution was injected intravenously and the animal sacrified after 24 hours. All the final experiments were carried out with activities of 1–2 millicuries. The intravenous injection was made after the animal had recovered from the anesthesia. A part of the solution was kept for determination of its activity. In all cases except 1 where we waited 80 hours, the animals were sacrified at the end of a 24-hour period.

For measuring the activities, a Geiger-Mueller counter with a glass window of about .01 cm. thickness was used. The number of counts was reduced by use of a scaling circuit in the ratio 8 to 1.

The teeth which were to be investigated were removed by a bone chisel and carefully isolated. To check the filling of the root-canal in the pulpless teeth, radiographs were taken and some ground sections were examined by the aid of a low power lens. The root-canal filling was found to appear well adapted to the wall of the canal. It is of interest to note that the measurements of the activity of different parts of pulpless teeth showed no difference between the 2 methods of filling the pulp canals. For our experiment each tooth was first divided by aid of a fret saw into 2 parts, the crown and the root. The edge of the enamel was taken as a border of the parts.

[2] We are indebted to Professor S. K. Allison and Dr. L. Slotin for preparing the labelled phosphorus.

The activities of crown and root parts of corresponding teeth were compared. For determining the radioactivity of the different tissues, the dentin was carefully separated from the enamel by aid of dental burs. This mechanical way of separation is possible because of the difference in hardness of the two materials. The enamel cannot be cut as easily as dentin by the bur and therefore remains as a shell, whereas the dentin is collected as a powder. From the outside of the root part a layer of about 1 mm. thickness was removed by the aid of an abrasive disc. By removing such an amount one can be sure that all the cementum is removed, although some dentin too is removed. It is very hard to separate these substances accurately since their macroscopic appearance is the same. The fact that by this method of separation some of the material was lost has no influence on the investigation since all the activities were related to the same weight (1 mg.).

Because of the unknown admixture of dentin to the cementum, the determination of the activity of the cementum is uncertain. An accurate value of the activity of the cementum was not important, especially since the metabolism of the cementum is probably not changed by removing the pulp or crowning the tooth and also since the cemento-dentinal junction is taken as a boundary of the tooth.

Finally the enamel and the root dentin of each tooth were pulverized and their activities measured. Since the measurements of all parts of the teeth of one dog were not made at the same time, the measured activities were related by using the half-life time of the phosphorus isotope.

RESULTS

If the teeth are divided into 2 parts, namely the crown and the root, some knowledge of the metabolism can be obtained by comparing analogous parts of corresponding teeth. In Table I, the averaged results for measurements on different pairs of teeth are shown. The numbers are the ratio between the activities of corresponding parts whose experimental conditions are indicated. The ratios are given in per cents.

From the data the striking result that, in spite of the removed pulp, phosphorus metabolism still exists, is evident. About one-

tenth of the radioactive phosphorus of a normal tooth was found in the crown of the pulpless tooth. The difference between the activity of the dentin of a normal and a pulpless tooth is much smaller in the root, as the phosphorus intake of the cementum is not affected by removing the pulp. This result is established with great accuracy since the experiment was often repeated by using different kinds of root-canal filling materials. As mentioned above, about the same results are obtained with the routine technique as with special techniques.

The next conclusion which follows is that when the coronal end of the pulp is injured, the phosphorus metabolism through the pulp is more or less inhibited (Table I). The metabolism of a tooth with an

TABLE I

Percent ratio of amount of P^{32} in experimental tooth to amount in corresponding control tooth of the same animal

CONDITIONS		CROWN	ROOT
Experimental	Control		
Removed Pulp	Normal	11	64
Removed Pulp with Metal Cap over Crown	Normal	12	67
Normal with Metal Cap over Crown	Normal	103	106
Injured Pulp	Normal	13	68
Injured Pulp with Metal Cap over Crown	Normal	12	70
Removed Pulp with Metal Cap over Crown	Removed Pulp	96	94
Injured Pulp	Removed Pulp	108	105

injured pulp does not differ markedly from that of a pulpless tooth, at least not during the first days after the injury. This result was confirmed by direct comparison of pairs of teeth, one of which had the pulp removed, the other pulp only traumatized.

From the data it appears, further, that in a normal tooth the phosphorus intake from the saliva is negligible in comparison with the intake through other pathways, i.e., the pulp and cemento-dentinal junction. The deviation from 100 per cent in these data is within the experimental error. This result is interesting since it was found that the saliva contains labelled phosphorus.

The comparison of lines 1 and 2 or 5 and 6 shows that the amount of phosphorus found in root and crown portions of a tooth

with the metal cap are about the same as that found when the enamel was not protected from the saliva. It thus appears that the phosphorus intake by the saliva is not important even in case of a removed or an injured pulp.

Table II shows the relative activities of different parts of the teeth under different experimental conditions. The numbers indicate in arbitrary units the activities of the same weight of tissue averaged over all the teeth. Since the dogs were injected with different doses to calculate the mean values, the values of the crown dentin of the normal teeth of each dog were equated and the values of all other parts correspondingly changed.

From Table II similar conclusions to those from Table I can be drawn. Furthermore, a more detailed picture of the metabolism of

TABLE II

CONDITION	CEMENTUM	ROOT DENTIN	CROWN DENTIN	ENAMEL	SALIVA
Normal	4.0	2.5	2.62	0.48	0.15
Normal with Cap		2.55	2.6	0.50	
Pulp Removed		0.2	0.24	0.1	
Pulp Removed with Cap		0.18	0.26	0.12	
Pulp Injured		0.24	0.28	0.13	
Pulp Injured with Cap		0.22	0.24	0.10	

different tissues can be obtained. It is seen that the cementum of a normal tooth contains more of the labelled phosphorus than the dentin, although, as explained above, the value of the cementum is not accurate because of admixture with dentin. Since the dentin contains less phosphorus than the cementum in the normal tooth, the given value for the cementum is too small. The values of the activities of the cementum are not shown for teeth with removed or injured pulp, for in these cases the values for the activity of the cementum would become still more inaccurate since the dentin shows much smaller activities. The real value of the activity of the cementum in all cases is certainly the same as in the normal tooth. It is seen that the dentin has about the same activity in crown and root portions in both normal teeth and teeth with removed or injured pulp.

It is interesting to observe that metabolism takes place even in the

enamel. This metabolism is present in normal teeth and in teeth with removed or injured pulp. From study of Table II it is apparent that the saliva is not responsible for the phosphorus intake of the enamel since the cap does not influence the activity. The given amount of the activity of the enamel of pulpless or injured teeth shows a smaller accuracy than all the other values because of its small content of active phosphorus. The activity of the enamel cannot be accounted for by small admixtures of dentin since the differences between the activity of the enamel is higher than could be explained by a small admixture. Furthermore, the table shows the activity of the saliva as compared with the same weight of tooth tissues.

Finally, it may be of interest to state the fraction of the total intravenous injection taken in by a tooth. Our data indicate that about one hundred-thousandth of the total amount injected goes into the dentin of a normal tooth. The amount going into the enamel of a pulpless tooth is only about a tenth of a millionth of the amount injected. The fraction of phosphorus going into the dentin of a normal tooth is slightly more than would correspond to the ratio of the weight of the dentin of the tooth to the weight of the animal. In case of the enamel of a pulpless tooth, much less labelled phosphorus from the injection enters the enamel than corresponds to the ratio of the weight of the enamel with the weight of the animal.

DISCUSSION

The results obtained by this study confirm the conclusions of previous investigators that phosphorus is steadily absorbed in adult teeth. Hevesy, Holst and Krogh therefore think it possible that a dissolution of tooth tissue may occur at one point and a corresponding precipitation of calcium phosphate occur at another area along the boundary between the circulatory fluid and the tooth tissue. However, as long as we can rely only on previous experiments with teeth whose pulp and odontoblasts were intact, we could not be sure whether the intake of phosphorus may be due to a dentin formation in so-called adult teeth. Because an individual has reached adulthood it does not follow that the odontoblasts may not continue to produce dentin (5).

Our results on pulpless teeth give a basis for further discussion of the idea of a replacement of minerals in adult teeth. In this con-

nection we must face the question as to whether the intake of phosphorus into the dentin of pulpless teeth is the same process as the apparently identical phenomenon in normal teeth. It is a generally recognized biological concept that a tissue lives only by virtue of its contained living cells. In these experiments we were studying *in vivo* an intercellular structure from which the cells had been removed.

Our results obtained with pulpless teeth differ from those with normal teeth only by the lower rate of intake. This is probably due to the smaller boundary between circulatory fluid and dentin. Hence these experiments should be evaluated in the same way as the corresponding experiments with normal teeth by Hevesy and others. The *a priori* distinction mentioned above between living and non-living tissues would be the only reason for limiting the use of the term metabolism to a ground substance without cells such as the dentin in pulpless teeth. Within the sphere of our experiments there is no evidence to justify such a distinction. Moreover, since the cell-free material is unchanged in structural pattern and chemical composition and still shows a sizable though decreased intake of phosphorus as compared with teeth having the cells, there is no avoiding the question that the turnover of mineral within the dentin in normal teeth may be independent of cellular activity. In other words, these experiments lead to the problem of the maintenance of intercellular structures. These structures, such as collagenous fibers, are chains and networks of micelles whose maintenance is basically due to physical forces (8). The old problem, whether and how far the intercellular structures remain dependent on the cells by which they have been built, now comes down to the question, whether and how far physical forces responsible for the micellar pattern are derived from or are connected with cellular activities. This question which is hardly older than the method used in our experiments cannot be answered on the basis of concepts not yet tested within the order of micellar structures and forces.

The results obtained by our experiments on pulpless teeth prove definitely that the cemento-dentinal junction is permeable to minerals. Although the pulp is the main source of supply, the intake of minerals by the way of the cementum is not negligible.

It cannot be decided whether or not dentinal tubules which extend

into the cementum are the anatomical substrate for this pathway of phosphorus intake. In any case, since there was no difference between crown and root part, the radioactive phosphorus is quickly distributed over the entire tooth no matter whether it is conducted through the system of dentinal tubules or along the fibers through the intertubular matrix.

Our findings concerning the presence of radioactive phosphorus within the enamel were anticipated by the results of Hevesy, Holst and Krogh, and are in harmony with the results of Hevesy and Armstrong, the only difference being that the amount of radioactive phosphorus recorded in our samples was considerably higher than Hevesy and Armstrong's "6.7 to 10 per cent of that of the dentin." Manly, Hodge and Van Voorhis (9) found "no more than traces of radioactive phosphorus within the enamel."

SUMMARY

1. Measurement was made of the intake of phosphorus by the teeth of adult dogs within 24 hours after an intravenous injection of an aqueous solution of radioactive sodium-phosphate. 2. The methods applied in this study are described. 3. Both normal teeth and the corresponding teeth on the opposite side of the jaw of which the pulp was either removed or injured were studied. 4. It was shown by this method that pulpless teeth continue to take up phosphorus by the way of the cemento-dentinal junction. About one-tenth of the amount of phosphorus taken up by normal teeth in 24 hours enters the pulpless teeth through the cementum. 5. The rate of phosphorus intake was the same in both pulpless teeth and teeth with pulp injured by a slight trauma. 6. When crown and root parts of the teeth were separatedly measured, no difference was found as to the amount of phosphorus contained within the dentin. 7. Since this is true of pulpless teeth where the phosphorus enters the dentin only on the root part via cementum, a quick distribution of the phosphorus throughout the dentin must take place. 8. By separating the 3 hard tissues of the tooth from each other, phosphorus was shown to have also entered the enamel. 9. If covered by an impermeable cap, the enamel nevertheless takes up the same amount of phosphorus. Therefore, the amount of phosphorus taken up by the enamel from the saliva

which contains radioactive phosphorus, is negligible compared with the uptake from the dentin even in pulpless teeth. 10. The question is discussed whether the intake of minerals by the tissues of pulpless teeth is the same process as the metabolic exchange of the same substances in normal teeth or if it is a purely physical adsorption or simple chemical combination.

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OSTEOSCLEROSIS OF JAWS[1]

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Areas of abnormally dense bone, of a variety of shapes and sizes, in the jaws are frequent enough findings in radiographs to demand attention and investigation as to their origin and as to the advisability of treatment. R. Kronfeld's[2] histological studies threw some enlightenment on findings of this sort, explaining them as a product of chronic periapical inflammation. There is no doubt that Kronfeld's investigations are correct inasmuch as these areas of bone condensation are occasionally located around the root ends of infected teeth. However, the experience of the radiologists makes it clear that such sizable areas are found also below and around vital teeth. This fact induces us to place limitations on the concept that sclerotic areas in edentulous jaws result in every case from infected teeth that have been removed. Our radiographic and histological studies indicate that consideration must be given to the fact that sclerotic areas in jaws may be adjacent to vital teeth. Another question is whether these zones of condensed bone surrounding vital teeth should be considered as having an etiological connection with the teeth or whether they develop independently of stimuli transmitted to the bone by the teeth.

DIFFUSE OSTEOSCLEROSIS

Osteosclerotic changes are characterized by an increase in the deposition of bone tissue leading to condensation of the bone and consequently a narrowing of the bone marrow spaces. Such changes may be diffuse or localized and can be seen in all parts of the skeletal system.

Diffuse osteosclerosis is the result of various toxic substances such as phosphorus and fluorine and it can be produced experimentally in

[1] Read at the meeting of the 19th General Meeting of the International Association for Dental Research, St. Louis, Mo., March 15, 1941. (*J. D. Res.* 20: 231, 1941.) (Received for publication April 7, 1941.)

[2] Kronfeld, Rudolf, *Histopathology of the Teeth.* (Phila., Lea and Febiger; 1939).

animals by administration of strontium over a long period of time. Osteosclerotic changes due to phosphorus are most likely caused by an excessive formative stimulus to the osteogenic connective tissue; whereas, osteosclerosis as produced by chronic fluorine intoxication resembles the condensation of bone found in healed rickets. An inhibition or a decrease of osteoclastic resorption is believed to be the cause of osteosclerosis following long standing administration of strontium.

Areas of dense brittle bone scattered over the skeleton were described in Albers-Schönberg's marble bone disease which may be congenital, hereditary or familial. It is possible that a lack or an absence of osteoclasts is responsible for the condensed bone, the spotted appearance of which is characteristic. However, the precise nature remains to be learned and, as yet, nothing is known concerning the role that the endosteum plays in this disease.

Certain diseases of the blood, such as leukemia, pseudoleukemia, anemia and polycythemia, are accompanied by sclerotic changes in bones. There is a reasonable probability that the areas of hyperplastic bone that diminish the marrow spaces are produced by the altered blood forming parenchyma and altered endosteum.

LOCALIZED OSTEOSCLEROSIS

Localized osteosclerotic changes of the bones may occur subsequent to syphilis, tuberculosis and inflammation within bone as a result of osteitis. In these instances, due to increased activity of the osteoblasts, the bone tissue assumes a striking density. While the rarefaction by osteoclasts markedly decreases or entirely ceases, osteoblastic deposition becomes predominant and new bone is formed to such a degree that marrow spaces are diminished to fractions of their former size. This condensation of bone may be so intensive as to give an ivorylike appearance and consistency to these areas, which radiographically appear as homogeneous dense and structureless masses. They may be sharply outlined or may have finger-like projections into the adjacent healthy bone tissue.

OSTEOSCLEROSIS IN THE JAWS

Since inflammation in jaws is, in the majority of cases, caused by infected teeth it is little wonder that sclerotic areas occur around the root ends of such infected teeth. Some of our radiographs disclosed

dense areas of structureless bone surrounding rarefying periapical processes. While these bone condensations extend deep into the inner part of the spongy bone, some may even extend farther and reach the alveolar crest (*fig. 1*). The periapical infection walled off by such condensed bone tissue seem to have been reduced in size and even partially filled in by bone trabeculae. However, the condensed bone, as revealed by radiographs, projects in various directions away from the zone of infection, thus affording evidence that these sclerotic areas developed outside of the region of apical infection and did not fully occupy the infected zones. We observed such a dense area around the root end of a molar extending far into the mandibular canal and even overlapping it. Thus it reached far beyond the boundaries of

FIG. 1. Condensed bone adjacent to periapical infection of a lower second molar

the apical process. Microscopic study of appropriate human specimens enabled us to study such radiographically observed structures in relation to their adjacent tissues.

Fig. 2 shows a bucco-lingual section through the region of the distal root of the lower first molar of a man, 33 years of age, who died of tuberculosis. The upper part of the pulp chamber contains abscesses, debris and necrotic tissue while the pulp of the apical region, as seen in other sections, is converted into granulation tissue which is rich in fibers and harbors a small, dense area of infiltration composed of polymorphonuclear cells, lymphocytes and plasma cells. The blood vessel walls are thickened and the nerves are fairly well preserved. The pulp tissue adjacent to the dentinal wall is loosely arranged due to edema. However, in spite of this derangement of odontoblasts,

connective tissue cells which replaced the odontoblasts layed down cell containing bone or cementum on the dentin. This deposition continued down to and around the apex and produced a striking hyperplasia of the cementum. Some of the apical fibers of the periodontal membrane are still discernible although their bundles are separated by chronically inflamed tissue. In *fig. 2* the periodontal membrane between the surface of the root and the alveolar plate is

FIG. 2. Bucco-lingual section through distal root of lower first molar of a man 33 years of age, who died of tuberculosis. Osteosclerosis (O), around root; periodontal membrane (PM) replaced by granulation tissue (GR).

wider and is replaced by an old fibrous granulation tissue containing strands of proliferated epithelium. Due to tipping of the tooth in the labial direction, there is an osteoclastic resorption of the labial alveolar plate still in progress, while on the lingual side there is deposition of new bone trabeculae upon the lingual alveolar plate with occasional deposition in the apical area. There is a very marked denseness of the supporting bone which projects diffusely into the

spongy bone of the body of the mandible and occupies a large part of the spongiosa. The abnormally narrowed bone marrow spaces contain chronically inflamed granulation tissue and are bordered by bone trabeculae that are coated with small osteoblasts. In spite of the severe inflammatory changes of the bone marrow, bone deposition alone is observed. The bone trabeculae containing large young cells show many incremental lines as a result of the rapid succession of bone deposition intervals. Deposits of new bone are found to be subperiostally located on the lingual border of the mandible as a product of a far reaching chronic inflammation. These osteophytes are characterized by their vertical direction in relation to the surface of the bone and by the presence of large osteocytes.

While the microscopic study of the case described above revealed a diffuse area of bone condensation resulting from a chronic periapical inflammation, a clearly circumscribed thickening of bone as a consequence of a chronic pulpitis without any noticeable involvement of the periodontal membrane is shown in the following case from which sections were taken of a girl, 16 years of age, who died as the result of a myocarditis (*fig. 3, 4, 5* and *6*).

In *fig. 3* formation of very condensed bone is shown around the root end of the lower left second premolar and the apex of the mesial root of the first molar. Both circumscribed areas of sclerotic bone extend to the mandibular canal and are associated with teeth the pulps of which are partially chronically inflamed, yet vital. *Fig. 4* shows a higher magnification of the root end of the second premolar with the adjacent bone, showing a very slightly inflamed pulp the structure of which has been converted into fibrous tissue and retains some fairly well preserved odontoblasts and dilated blood vessels. The dilatation of the blood vessels is a reaction to chronic inflammation present in the coronal pulp. In those areas where the pulpal surface of the dentin is not outlined with odontoblasts there is a deposition of bone or cementum in progress. The periodontal membrane has retained its normal structure and does not contain any inflammatory cells. The presence of dilated blood vessels here is remarkable. The bone marrow spaces the contents of which have been converted into more or less dense fibrous tissue without inflammatory cells are narrowed by the new formation of bone upon their walls (*fig. 5*). This condensed area ex-

FIG. 3 FIG. 4

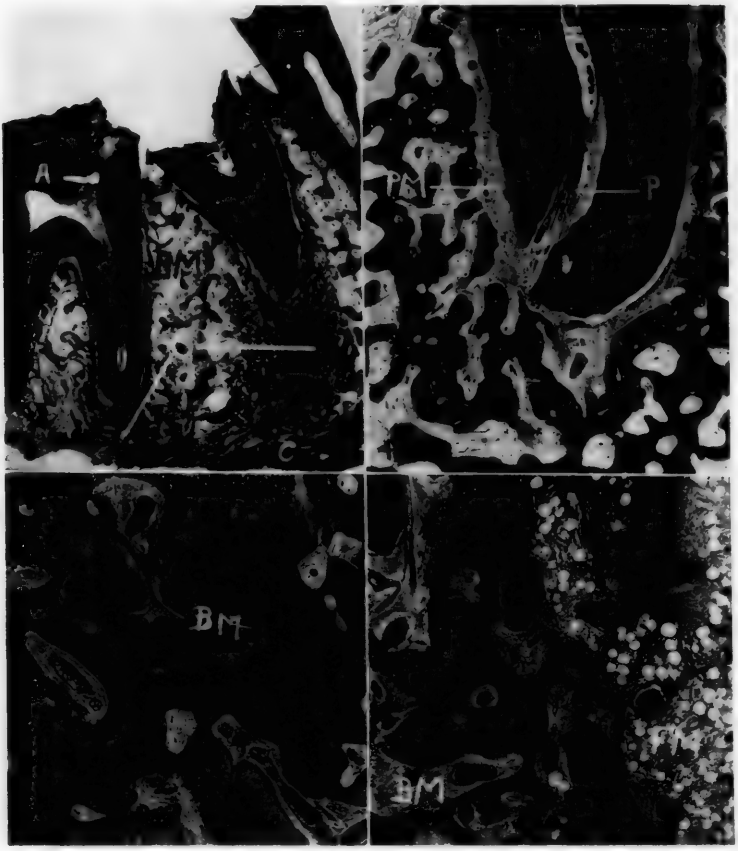

FIG. 5 FIG. 6

FIG. 3. Mesio-distal section through lower left second premolar and first molar of girl 16 years of age, who died of myocarditis. Osteosclerosis (O), surrounding premolar root and mesial molar root extends to mandibular canal (C). Fibrosis of bone marrow (BM). Abscess (A), in molar pulp walled off; rest of pulp and periodontal membrane chronically inflamed.

FIG. 4. Higher magnification of root end of premolar of *fig. 4*. Pulp (P), converted into fibrous tissue with dilated blood vessels. Periodontal membrane (PM) of normal structure with dilated blood vessels.

FIG. 5. High magnification of sclerotic bone area of *fig. 4*. Narrow spaces of fibrous marrow (BM).

FIG. 6. High magnification of boundary between sclerotic bone with narrow spaces of fibrous marrow (BM), and normal bone with wide spaces of fat marrow (FM).

tends to the mandibular canal and is fairly definitely defined. The lack of resorption lines and the presence of incremental lines in these bone trabeculae is evidence that osteoclastic resorption did not occur and moreover, that deposition alone occurred. The marrow of the condensed bone is converted into a loose fibered marrow. However, fat cells are present in the marrow spaces at the boundary between sclerotic bone and normal bone (*fig. 6*).

In another case we found osteosclerosis of the alveolar bone having no relationship to the pulps of the neighboring teeth. The radiograph (*fig. 7*) shows a structureless compact bone making up the entire supporting bone between the right lower canine and the first premolar. Both teeth gave positive vitality tests. While the periodontal membranes of the adjacent teeth do not reveal any striking changes, the crest of the dense interdental bone is markedly hollowed out by resorption that proceeded vertically. On account of the absence of any microscopic studies of this case we can only speculate as to the development of this sclerosis. Since there is noticeable resorptive inflammation involving the crest of the alveolar process, we feel justified in associating the condensation of bone to this chronic inflammation called marginal periodontitis. This conclusion is based on our knowledge of the relationship between bone sclerosis and inflammation and is more acceptable than the supposition that the sclerosis was etiologically connected with an unknown factor and had been in existence before the resorption of the crest had begun.

Our conclusion drawn from this rare radiographic finding of sclerosis of the interdental bone is supported by the microscopic study of an accidental finding in the mandible of a woman, 28 years of age, who died of tuberculosis. In *fig. 8*, sclerotic changes of the alveolar and supporting bone between the lower left canine and the first premolar are demonstrable. Both teeth were steadily drifting toward the median line, as evidenced by the presence of many incremental lines in the distal portions of the alveolar plates of both sockets. The supporting bone is replaced by a very dense bone formation containing bone marrow spaces, the contents of which have mostly been converted into fibrous tissue. The tip of the alveolar crest is covered with small round cells as a result of the subepithelial chronic inflammation of the gingiva. Furthermore, accumulations of inflammatory cells, which

are a consequence of this inflammation, can be seen within the fibrous marrow. Therefore, these findings induce us to regard the condensation of the interdental bone as the result of a mild chronic inflammation in an individual whose constitution was of a peculiar nature. A noteworthy finding and one that complies to the same point of view is the fact that such a sclerosis of interdental bone, however of less extent, is found between the adjacent teeth as a response to a fairly deep pocket formation of the gingiva.

FIG. 7

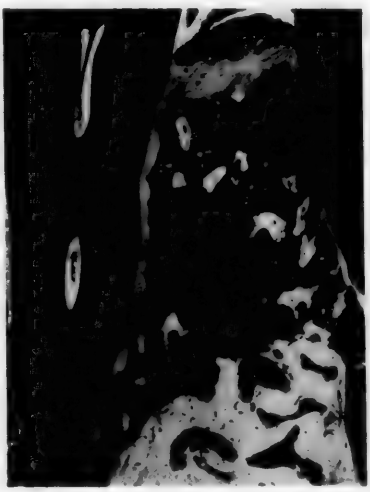

FIG. 8

FIG. 7. Sclerosis of the interdental alveolar bone between vital canine and vital premolar. Note resorption of alveolar bone of canine.

FIG. 8. Mesio-distal section through lower left canine and first premolar of woman 28 years of age. Both teeth were vital. Sclerosis of interdental alveolar bone.

While there is a reasonable probability that sclerotic changes of the type described above are casually connected with a reaction of the bone tissue to a mild chronic inflammation of the pulp or of the gingiva in an individual whose constitution is of a peculiar nature, we have a radiograph on hand that points to the fact that condensations of the jaw may be caused also by mechanical irritation. Unfortunately, however, there was no material available for microscopic study and this problem is still open for discussion and explanation. In *fig. 9*,

the radiograph shows the bridged lower left canine, first premolar and second molar. These teeth were vital. The area extending from the root end of the first premolar to the mesial root of the second molar is occupied by a cloud-like condensation which is fairly well circumscribed around the premolar without interfering with its periodontal

FIG. 9

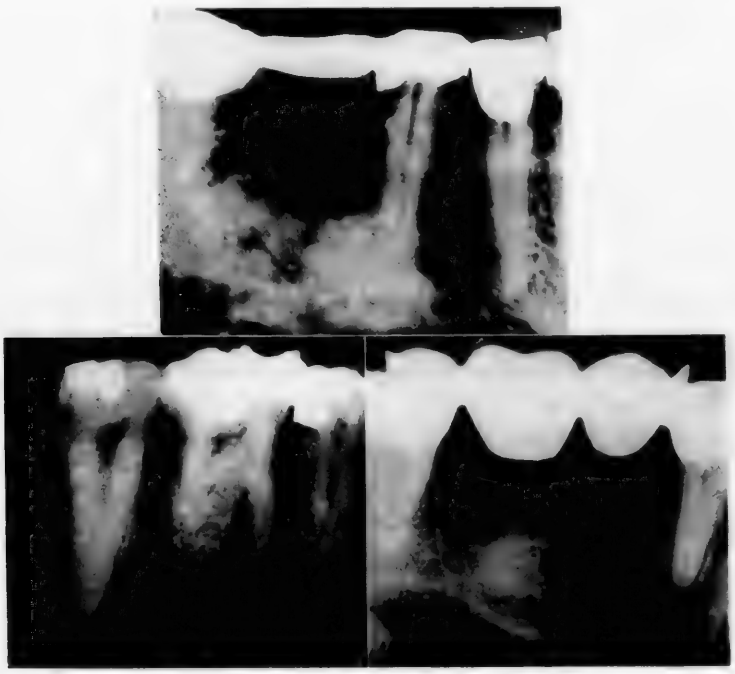

FIG. 10 FIG. 11

FIG. 9. Diffuse sclerosis around bridged vital lower second premolar and second molar.
FIG. 10. Radiograph taken before extraction of second premolar and first molar.
FIG. 11. Radiograph taken 5 years after extraction of second premolar and first molar of the patient of *fig. 10*. Note sclerosis.

membrane. The adjacent bone structure does not reveal any changes. This distribution of the very dense bone tends to support the assumption that the exciting factors are to be attributed to the vital teeth rather than to the conception that the sclerotic areas are associated with the extracted teeth. From this

consideration an attempt may be made to attribute this condensation of bone to a chronic mild irritation caused by the stresses upon the fixed partial denture transmitted to the bone, the constitutional makeup of which is peculiar, thus stimulating osteoblastic activity.

Finally the question arises as to what is the origin of those sclerotic areas that are located in edentulous jaws or in edentulous areas. On the basis of our studies they may be associated in some cases with pulpless or more frequently with vital teeth. Radiographs (*figs. 10* and *11*) indicate that even the extraction of a vital tooth without periapical involvement may result in a formation of sclerotic bone in the socket in spite of the fact that an uncomplicated healing occurs. *Fig. 10* is a radiograph of the maxillary right second premolar and 2 molars taken at the time of extraction, 5 years ago. Because of neuralgic pain in this region the premolar and the anterior molar were removed but the pain did not subside. An undisturbed healing process followed the extraction of these teeth and a fixed partial denture was inserted. A radiograph taken 5 years later revealed sclerotic bone in the place of the former anterior molar (*fig. 11*). Although the healing was undisturbed by any noticeable inflammation, an abnormally dense apposition of bone occurred resembling the hyperplastic scar tissue of the keloid.

COMMENT

The radiopaque areas in the lower jaws of human beings are, according to our microscopic examinations, more or less clearly circumscribed condensations of bone trabeculae confined within the mandible. The increased density of the trabeculae is from the inside to the outside of an area without any direct activity of the periosteum. It extends far from the focal area, overstepping possible extension from a periapical focus. This statement is important in considering the development of these sclerotic zones. All of the newly formed bone is well calcified and covered by narrow osteoid zones lined with osteoblasts. We were not able to demonstrate any osteoclasts in any of our sections despite the fact that irritating forces brought about changes that involved the bone marrow and converted it into a loose fibrous tissue. This fibrous tissue located in extremely reduced bone marrow spaces was almost always entirely free from round cell in-

filtration. There was only one case observed in which chronic inflammation of the bone marrow was noted and even in this instance osteoclastic activity was lacking while deposition of new bone extended over and somewhat beyond the entire lesion. This case was the only example of sclerosis that was without doubt related to periapical chronic infection. Our microscopic studies of other periapical sclerotic areas disclosed that these areas were causally associated with partially inflamed yet vital pulps and with no particular involvement of the periodontal membrane. Furthermore, in our radiographic and microscopic studies we found sclerotic areas of the interdental alveolar bone resulting from a marginal periodontitis. Since it is known that deposition of bone is stimulated by intermittent stresses occuring over a long period of time, we do not hesitate to associate sclerotic bone with the vibratory forces exerted upon a fixed partial denture.

An immediate consideration arises which must be explained in order to determine the etiology of these dense bone formations: What is the factor involved in the genesis of these lesions, since in the majority of people who have infected vital teeth, marginal periodontitis, or tooth extractions, there are no sclerotic areas?

There are 2 facts that have influenced the interpretation that we are about to advance. Undoubtedly, the excessive formation of bone in the cases that we studied is due to increased activity of the osteoblasts of the endosteum. Further, the extent of the involved areas far exceeds the limits that a periapical infection could directly affect. The sclerotic bone of the jaw is the result of a peculiar type of sensitivity on the part of certain individuals to the effects of an irritation that occurs over a long period of time. It appears that this chronic irritation which is able to convert the bone marrow into fibrous tissue and which is able to so excessively stimulate the osteoblastic activity of endosteal cells is produced by mild inflammatory changes. The stimulus is of necessity of a mild nature because there are no evidences of osteoclastic resorption. We believe that the peculiar sensitivity strongly resembles the sensitivity of the connective tissue of certain individuals to keloid formation. Osteosclerosis of jaws represents a similiar peculiarity of reaction on the part of the endosteal osteoblasts. Being nothing more than hyperplastic tissue the localized osteosclerosis of jaws does not require any treatment.

VITAL STAINING OF GROWING BONES AND TEETH WITH ALIZARINE RED S[1]

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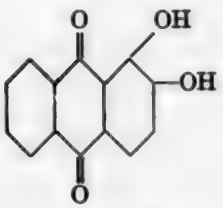
The increasing use of alizarine Red S as a vital dye prompted this report. In contrast to the method of prolonged madder feeding which gives diffuse effects (2, 5, 13), each injection of alizarine Red S produces a sharp red line in the incremental zones of dentin, cementum and bone (14, 15) that are growing and calcifying at the time. The distance between 2 successive injections thus represents the amount of apposition that has occurred during the intervening period. Sharp effects were not found in the enamel.

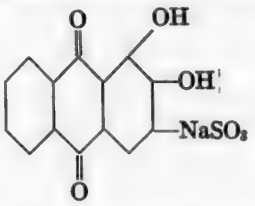
History. Robiquet and Colin (8) in 1826 reported alizarine to be the principal staining ingredient in madder, which was used in the dyeing of cloth by the early Egyptians. It was synthesized in 1869 (8) and Perkin and Story (18) synthesized 3-iodo-alizarin in 1931. By vital staining with alizarine Red S, studies have been made on bones and teeth of various species ranging from fish to monkey (1, 7, 10, 14, 17, 20). Alizarine has also been used as a non-vital stain in the Spalteholtz method of clearing (25), differential staining of bones (7, 17), and in ground sections of enamel (11).

Chemistry. It is important to distinguish between alizarine Red S and other members of the alizarine group. The general formula of the latter is:



[1] This study was made with the aid of the Carnegie Corporation of New York and Mead Johnson and Company. (Received for publication April 17, 1941.)

1–2 dihydroxy anthra-quinone, and is less consistently effective in vital staining. It exists in madder as a glucoside, together with purpurin and purpurin 3-carboxylic acid. Alizarine Red S is:



It is the sodium salt of alizarine mono-sulfonic acid, 1–2 dihydroxy 3-sodium sulfonic anthra-quinone.

Alizarine Red S although classified as an acid dye, like alizarine, is not itself a dye, but it forms insoluble pigments or lakes with the metallic hydroxides, which are deposited in the process of treatment with alizarine Red S. It exists as a colloid in the presence of serum and spreads poorly in a gel of high percentage (24). Shimidzu (24) also found a positive correlation between the very slight capacity of the dye to penetrate the placenta and the colloidal nature of the dye in the serum. Cameron (7), however, stained the bones of the fetus by injecting the mother with large amounts of alizarine.

The sodium salt of alizarine-3 sulfonic acid is obtained by the direct sulfonation of alizarine. The sulfonic acid group makes it much more soluble than alizarine (1–2 dihydroxy anthra-quinone). Alizarine is sparingly soluble in water, but readily soluble in ether, fixed oils and alkaline solutions.

Alizarine Red S, like many hydroxy anthra-quinone derivatives, is used as an indicator. The color changes with our sample occur between pH 3.7 (yellow), 4.2 (pink), 5.5 (red), 6.8 and above (purple).

Mode of Action of Alizarine Red S. Gottlieb (10) believes that alizarine has a specific chemical affinity for calcium. Cameron (7) states that the tinctorial effect in the hard tissues is due to the property of alizarine to form a dye lake with calcium, and that it is a nearly specific dye for calcium. Proell (20) states that alizarine stains the free calcium. Pollack (19) reports that when a solution of sodium alizarin sulfonate is injected into the ameba the presence of calcium in protoplasm can be demonstrated by the formation of red insoluble bodies which he believes are calcium alizarinate.

Richter (21) conducted in vitro experiments by staining fresh bones (of young rats) incubated at 37° in buffered solutions (pH 4.6, 5.7) of alizarine and other related dyes. His results were obtained after 3 hours and were similar to those obtained in vital staining.

To follow the tinctorial effects of alizarine Red S in vitro, an artificial bone matrix was prepared by one of us (M.H.) by extracting mucoid from a muco-collagen (ox tendon Achilles) and adding salts similar to those found in bone. The results showed that calcium is not necessary to the fixing of the dye effect, but rather the change of the medium from a muco-collagenous one to that of collagen. There was a gradual transition of the alizarine Red S coloration from a yellow to a purplish-red (increase in pH). The resulting medium manifested itself in bands composed of alternating purplish-red and pink stripes, indicating a distinct alkaline and an almost neutral phase respectively in regard to $(H)^+$. It thus appears that alizarine Red S has a selective staining effect upon the calcifying or calcio-receptive zone of the collagenous matrix where the calcium salts are being deposited.

Preparation of Solution. A filtered saturated solution of alizarine Red S (2 per cent) in 0.45 per cent sodium chloride was found to be isotonic with mammalian blood (Beckmann freezing-point determination).

Alizarine Red S of color index 1034, obtained from the Coleman and Bell Company, proved to be most consistently effective. The solution, which has a pH of 3.7 (quinhydrone) is stable at room temperature and maintains its effectiveness for at least 1 year. Boyle, Bessey and Howe (4) have prepared a 5 per cent solution with a pH of 7.

Dosage. The effective dosage for the rat, rabbit, guinea pig, cat, dog, monkey and human infant ranges approximately from 50–100 mg. per kg., depending on the age, weight and species.

New-born rats were injected successfully with 0.2 cc. of a 2 per cent solution, or approximately 150–200 mg. per kg. White rats weighing 100–200 grams require ½–1 cc. of a 2 per cent solution per injection. Younger rats are thus more resistant to higher pharmacologic doses by weight than older animals. It is possible that this may be related to the differences in the growth and calcification activities of the younger and older animals.

Method of Administration. Injections of alizarine Red S solution are superior in their effects to the feeding of alizarine powder. Intra-

peritoneal injections were found to be most effective and least toxic. Intravenous injections may be complicated by thrombosis of the veins at the site of injection and present the danger of emboli. Subcutaneous and intramuscular injections are effective but are complicated by sloughing of the tissues at the site of injection.

Transitory Effects. The activity of the animal is diminished for several minutes following the injection. The red coloration of the urine (and feces) may be seen within one hour after administration and may continue for several days. The duration of the effect depends on the age and dosage. The dye (which may be intensified in color by the addition of a few drops of N/10 NaOH) is also found in the secretion of the salivary and lacrymal glands and in those of the trachea. Cameron (7) reports transmission of the dye through the placenta, with resultant staining of the fetus.

While an injection of alizarine Red S (100 mg. per kg.) does retard the gain in body weight in rats, the weight curve tends to approach the normal level (*fig. 1*) and gains normally following subsequent injections. Despite the temporary loss or retardation of gain in body weight, the normal rate of apposition of bone and dentin is not affected (15).

Toxicology. Alizarine Red S when administered intraperitoneally at a dose of 100 mg. per kg. produces no significant toxic effects. Gross and microscopic examination of the vital organs shows no pathologic alterations. The temporary slight arrest in gain of weight is soon recovered (*fig. 1*). However, toxic effects are produced when higher doses are administered frequently (3 times a week) for prolonged periods. In these cases microscopic alterations were observed in the kidney epithelium, the severity of the effects depending upon the dosages employed. In acute experiments in dogs the initial intravenous injection of 10 cc. of our alizarine Red S solution produced no changes in blood pressure or respiration. Higher doses, however, resulted in a rapid marked drop of blood pressure, increase in heart rate and concurrent dyspnea.

Methods of Preparation and Study. Specimens fixed in 95 per cent alcohol or in neutral 10 per cent formalin solution will retain the color effects indefinitely.

For gross studies, the soft tissues are removed and the stained sites are examined.

For microscopic studies, ground sections (25 to 50 microns thick) are prepared, since the staining effects are lost by the action of the acid used in preparing decalcified sections. The method of McLean and

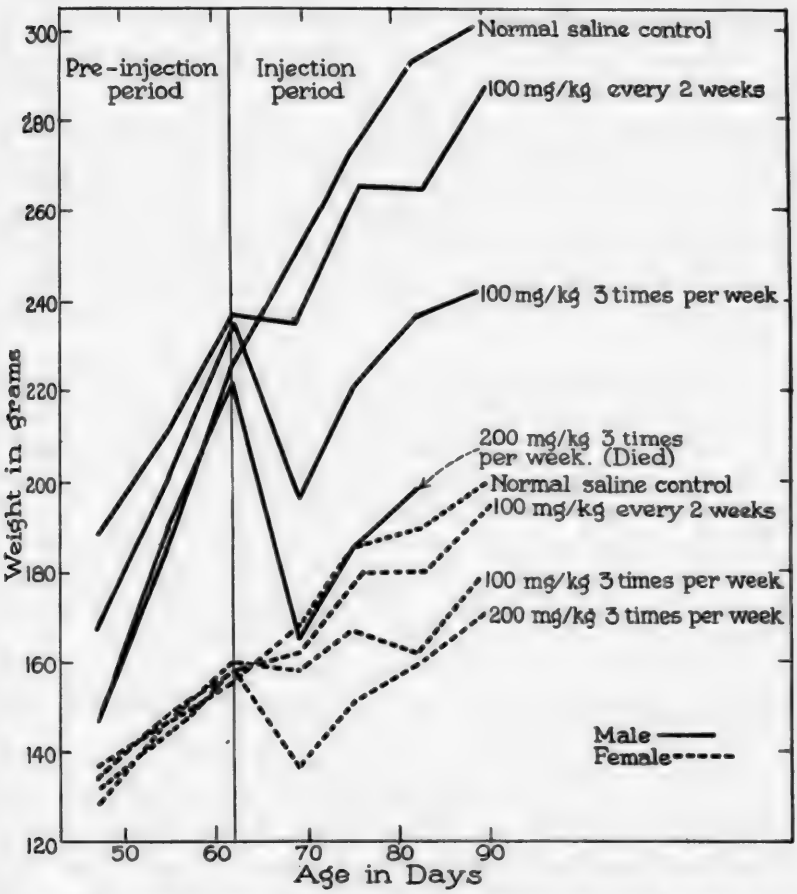


FIG. 1. Effect of various multiple doses of intraperitoneal injections of alizarine Red S on weights. Curves represent average weight of each group of three albino rats. Doses of 300 mg./kg. within 24 hours proved fatal. Animals weighed weekly.

Bloom (29) of cutting thin sections of bone without decalcification facilitates serial studies.

After gross dissection, the desired plane is obtained by use of a saw.

Tangential cuts should be avoided. The specimen is ground to the desired thickness by hand or by means of a motor-driven moist carborundum stone. The grinding of a fragile section is facilitated by fixing it on a cork with cellulose cement. The section is then cleaned, dehydrated, cleared in xylol and mounted.

Permanent Gross and Microscopic Effects. Skeletal preparations show generalized or localized coloration, depending upon the active sites of growth of the animal at the time of the administration (22). When the injections are given sufficiently far apart the effects may be seen as sharp lines even to the naked eye. The direct or indirect method may be used. In the direct method the vitally stained areas are studied. In the indirect method, which is best suited for studies of rapid growth, the animal is allowed to live for some time after the injection and the unstained areas that are subsequently apposed are analyzed.

Ground sections show sharp red lines which may be studied under a dissecting microscope with reflected light. Under higher magnifications and strong transmitted illumination the red lines (5–20 microns in width) are readily counted and the distance between them can be measured with a micrometer eyepiece.

The most immediate effect of the vital staining is the pink coloration of the narrow border of dentin or bone that is calcifying at the particular time of the injection. Dentin or bone calcified previous or subsequent to the administration is not stained. The effect is seen within 15 minutes after the injection. Animals that were allowed to live 1 year or longer after vital staining still showed the alizarine effects in the dentin and bone.

Application of Vital Staining with Alizarine Red S in Experimental Studies. The alterations in the rate of dentin growth in deficiencies of vitamins A (23), C (4) and D (16), magnesium (9), in endocrinectomies (26, 27) and in hibernation (28) have been reported. The use of this method for the biologic assay of vitamin A or C have been suggested. Alizarine has also been used in the studies of pathologic calcification, such as fractures and callus formation (6, 7), and pathological calcification (7, 12). Studies on the rate of formation of calculi should be facilitated by the alizarine method.

Vital staining with alizarine Red S offers a possible method of assay-

ing growth-promoting or growth-inhibiting substances. Further application of this method in studies of appositional growth and of physiologic and pathologic calcification are being conducted. It is suggested that a preparation of alizarine Red S which contained radioactive or radiopaque (18) groups would further facilitate its effectiveness in experimental studies.

SUMMARY

An intraperitoneal injection of a saturated solution (2 per cent) of alizarine Red S at a dosage of 50–100 mg. per kg. produces a sharp red line in the dentin and bone calcifying at the time. This method permits the measurement of the rate of growth of calcifying structures in normal and pathologic conditions in various experimental animals. Effective doses can be given without significant toxic complications.

We wish to take this opportunity to thank Drs. W. H. Welker, R. Ingraham and M. Berg, of the University of Illinois College of Medicine for their valuable guidance, and Mr. James Gold who assisted in the study of toxic effects.

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EFFECTS OF MALE SEX HORMONE ON GINGIVAE AND ORAL MUCOUS MEMBRANES[1]

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This study deals with the effects of the male sex hormone on the gingivae and oral mucous membranes of 6 rhesus monkeys, and a series of men and women, under treatment for such conditions as genital hypoplasia or persistent uterine-bleeding. Two of the monkeys were normal males, 2 castrated males, and 2 normal females. All were in the class of sub-adults. The hormone was injected intramuscularly. Tissue biopsies were taken pre-experimentally as well as at autopsy. Biopsies were also taken of the gingival tissue of the human group.

The animals studied ranged in weight from 3500 to 4750 grams. Testosterone propionate[2] was injected in daily doses varying from 5 to 25 mg. The total dosage was between 400 and 500 mg. in the "heavily" treated animals as compared with 100 to 200 mg. in others. The period of experimentation covered 18 days in the case of shortest duration, and 32 days at the longest. The human group was treated over longer periods, and the resultant total dosage was accordingly higher. Details of treatment are listed in Table I. For illustrative purposes, photomicrographs of sections of tissue from a male and female human and a male and female monkey were selected.

The parenteral injection of the male sex hormone generally has a stimulating action on the gingivae and oral mucous membranes. Grossly there is a slight thickening of the gingivae. This change is not impressive. Evidences of inflammation are either minimal or absent. This observation applies to both the human and animal groups. Microscopically, there are evidences of activity. The stain-

[1] Read at the 19th General Meeting of the International Association for Dental Research, March 15–16, 1941, St. Louis, Mo. (*J. D. Res.* 20, 243, 1941.) (Received for publication April 28, 1941.)

[2] Schering's Oreton.

ing reactions are uniformly deep. The keratin layer is increased in size, becomes firmer in that a more definite layer is seen, and it does not strip off as easily in sectioning. Hyperkeratinization is prominently emphasized by the appearance of a layer of keratin, as much as 6 or 7 cells deep, on the areolar gingivae and cheek mucous membrances, where keratin is not normally seen (*figs. 2, 3, 4,* and *8*).

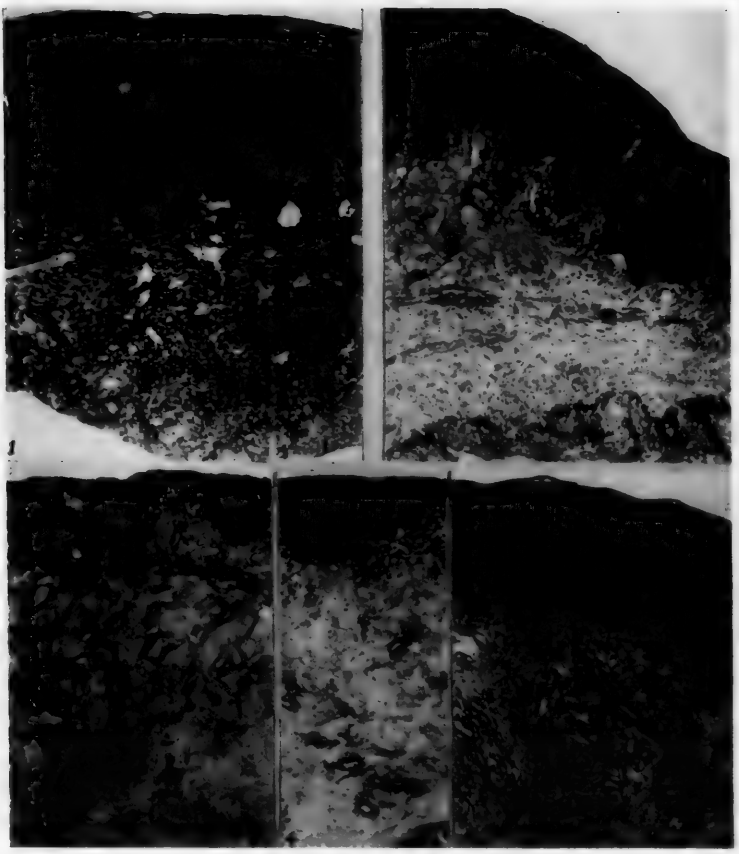
TABLE I

Effect of treatment with the male sex hormone on the gingivae and oral mucous membranes

MONKEY NO.	SEX	WEIGHT (GRAMS)	OPERATION AND TREATMENT	EPITHELIUM							STROMA CONNECTIVE TISSUE COMPACTNESS
				Keratin layer				Prickle cell layer			
						Pearls	Curls	Hyperplasia			
				Alv.*	Ar.**	Alv.	Ar.	Alv.	Ar.	Pegs broadened and squared	
449	0	3850	405 mg. Oreton in 32 days. Treatment 4 days following castration	+++	+++	0	+	++++	++++	++++	++++
443	0	4000	160 mg. Oreton in 32 days. Treatment 4 days following castration	+++	+++	0	++	++	+++	++	++
474	0	4000	Oreton—135 mg. in 18 days	+++	+	0	+	+	+	+	+
476	0	4750	Oreton—220 mg. in 30 days	++	+	0	+	+	+	±	+
472	0	4600	Oreton—490 mg. in 29 days	++++	++++	−	+++	+++	+++	++	++++
473	0	3500	Oreton—125 mg. in 29 days	++++	++++	0	+++	++	++	++	++

* Alv. = alveolar.
** Ar. = areolar.
0 = Negative. − to ++++ = Degree of change.

The appearance of tufts in the newly formed keratin layer on the mucous membranes is striking evidence of the active growth of the tissue (*fig. 3*). (Table I, "curls"). The prickle cell layer undergoes emphatic changes in the markedly increased cellularity, the crowding of the nuclei in the germinal layer, and the squaring and blunting of the epithelial pegs (*figs. 2* and *8*). Hyperplasia of this layer is further evidenced by the hyperchromatism of the nuclei, their prominent membranes and nucleoli, and increased mitosis up to 4 or 5 times normal.



FIGS. 1–5

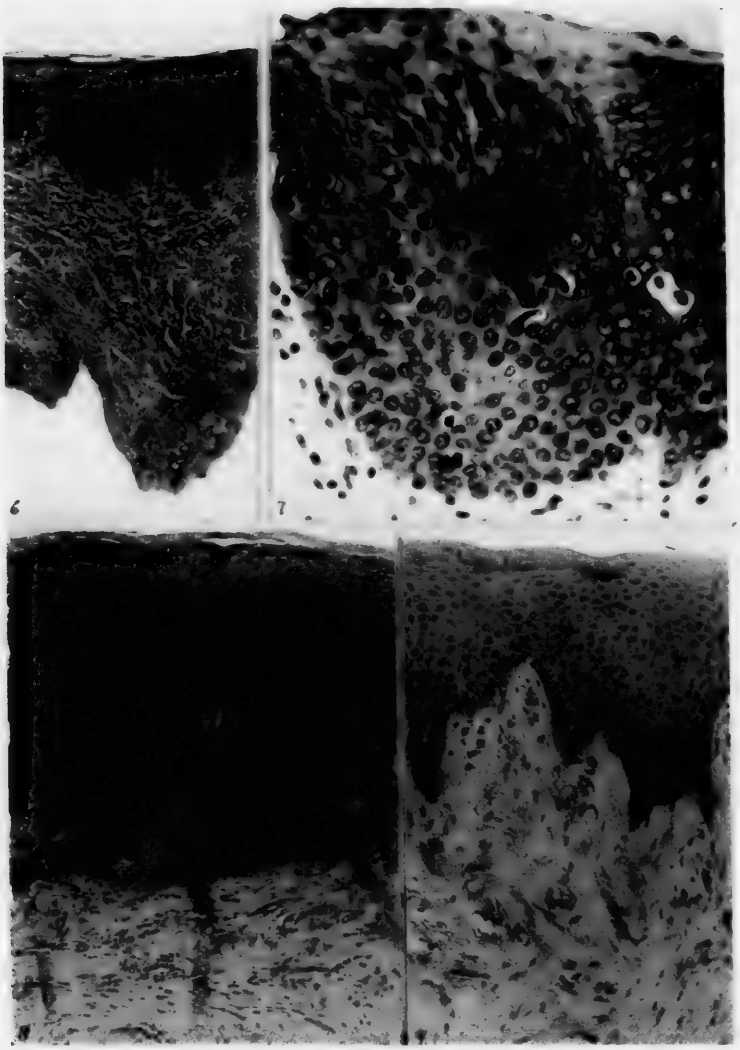
FIG. 1. My #472. Female. Wt. 4600 gms. Pre-experimental upper left #3 papilla. Alveolar gingiva near crest of interdental papilla. Animal having menstrual bleeding at time biopsy was taken. Note shape of epithelial pegs. Inflammatory exudate is more intense than usual. (Orig. mag. × 150)

FIG. 2. My #472. Same as *fig. 1*. Upper left #2 papilla. Post-mortem biopsy. Alveolar gingiva, comparable area to *fig. 1*. Treatment: 490 mgm. Oreton (testosterone propionate) in 30 days. Note broadening and squaring of epithelial pegs; increased number of nuclei; hyperkeratinization; also change in connective tissue. Corium is thickened and somewhat homogeneous in structure. Increase in collagenous bundles and active fibroblasts. (Orig. mag. × 150)

FIG. 3. My #472. Same as *figs. 1* and *2*. *Areolar* gingiva near muco-buccal fold. Note hyperkeratinization (keratin not normally found in this area); hyperplasia of both epithelium and connective tissue. (Orig. mag. × 150)

FIG. 4. My #472. Same animal as *figs. 1, 2*, and *3*. Section from cheek. Changes similar to those described in *fig. 3*. (Orig. mag. × 150)

FIG. 5. My #449. Male. Wt. 3850 gms. Pre-experimental upper left #2 papilla. Alveolar gingiva near crest of interdental papilla. (Orig. mag. × 150)



FIGS. 6–9

FIG. 6. My #449. Same as *fig. 5*. Upper left #2 papilla (regrowth of excised pre-experimental papilla). Treatment: *Castrated*; 385 mgm. Oreton (testosterone propionate) in 31 days. Note hyperplasia of both epithelium and connective tissue and broadening and squaring of epithelial pegs. (Orig. mag. × 150)

FIG. 7. My #449. Same as *figs. 5* and *6*. Note prominent nuclear membranes and nucleoli; frequent mitosis. (Orig. mag. × 560)

FIG. 8. Human, female, age 35 years. Upper left #3 interdental papilla. Alveolar gingivae. Treatment: 745 mgm. Oreton (testosterone propionate) in 84 days for pernicious uterine bleeding. Note hyperkeratinization; increase and crowding of nuclei in germinal layer; squaring of epithelial pegs; increase of cellularity and density of connective tissue. (Orig. mag. × 150)

FIG. 9. Human, male, age 30 years. Upper left #2 interdental papilla. Alveolar gingivae. Medical Diagnosis: Froehlich disease. Treatment: 2125 mgm. Oreton (testosterone propionate) in 33 weeks. Note increase in number of nuclei in both epithelium and connective tissue and increase in density of connective tissue. (Orig. mag. × 230)

Definite changes take place in the corium. Hyperplasia is marked. Large collagenous bundles comprise the greater portion of the tissue. A moderate increase is noted in the number of active fibroblasts scattered throughout. Many fibrocytes, apparently devoid of activity, are also seen between bundles. The extensiveness of the collagenous bundles account for the homogeneous, hyaline-like appearance of the connective tissue and its increase in thickness (*figs. 2* and *3*). Vascularity is slightly more pronounced. Edema is present in isolated areas and inflammatory exudate is minimal.

The most extreme effects were seen in those animals receiving the largest doses. The hormone reacted equally on male and female and castration did not produce a deviation. The humans were similarly affected in a degree proportionate to the dosage.

A comparison of the effects on the gingival tissues of the male sex hormone and the estrogenic hormone is pertinent. Both are tissue stimulators, non-specific in type. Both produce hyperkeratinization, and hyperplasia of the prickle cells and collagen. Both tend to reduce inflammatory reactions in the corium. The following differences have been noted. Epithelial hyperplasia with the estrogenic hormone is manifested by a downgrowth of the epithelial pegs and generally an increase in the width of this tissue. The usual epithelial peg pattern, however, is maintained. With testosterone propionate, hyperplasia of the epithelium is manifested in the same way but with a change in the peg pattern (squaring and blunting). The collagen change is more marked with the use of testosterone propionate, while the epithelial change is more marked with the use of the estrogens.

The changes following testosterone propionate resemble those following the use of progesterone (to be reported later). The latter were of lesser degree.

CONCLUSION

The effect of the male sex hormone on the gingival tissues of monkeys and humans is hyperplasia of the epithelium and connective tissue, especially marked in the latter.

EXPERIMENTAL STUDIES ON THE TOXICITY OF PROCAINE[1]

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The toxic effects of the local anesthetics are due mainly to three actions, the most important being a depression or paralysis of respiration. This is the usual cause of death in procaine poisoning. The second effect is a depression of the circulation resulting in a fall in blood pressure. The third effect is on the central nervous system, possibly the cerebral cortex, and is manifested by the production of convulsions which may be followed by a state of depression.

Procaine poisoning usually results in respiratory paralysis followed by delayed cardiac failure which many workers think is due to asphyxia rather than to direct depression of the heart, since the animals can be revived in most cases by prompt administration of artificial respiration, as shown by Tainter and Throndson (1). Potassium cyanide, in doses which markedly stimulate respiration has been demonstrated by Knoefel, Herwick, and Loevenhart (2) to have very little effect in the respiratory depression after procaine administration. They concluded that respiratory stimulants were of no value in the therapy of intoxication from local anesthetics. However, metrazol has been successfully used by Zipfe and Hoppe (3) to counteract procaine in white mice and cats when the 2 drugs were given simultaneously.

This paper deals with the acute toxicity of procaine in the dog and possible antidotal agents, together with observations on the development of tolerance. In all the experiments to be described, the term "procaine" refers to procaine hydrochloride.

[1] From a thesis submitted in partial fulfillment of the requirement for the Ph.D. degree, University of Chicago. (Received for publication April 7, 1941.)

[2] Now at College of Physicians and Surgeons, San Francisco, Cal.

PROCEDURE AND RESULTS

Acute Toxicity. Lethal doses were determined on dogs anesthetized with 2 cc./kgm. of paraldehyde orally. The vagi were sectioned, blood pressure recorded in mm. Hg. and respiratory movements recorded by means of a pneumograph and tambour. Repeated doses of increasing size of 2 per cent procaine were injected intravenously at a rapid rate until the lethal dose was reached. At least 20 minutes were allowed to elapse between injections to minimize cumulative effects, as Hatcher and Eggleston (4) have pointed out that a fatal dose of procaine is destroyed in 20 minutes by the liver. In 20 dogs, the mean lethal dose was 9.7 ± 3.8 mgm./kgm. ($s.d._m = \pm 0.85$). There was an abrupt fall in blood pressure and depression of respiratory rate and amplitude as has been observed previously. In most cases, the circulation was maintained for a few minutes after respiratory movements had ceased. This was in accord with Isenberger's (5) results after intravenous injections of procaine in the dog. On the other hand, Knoefel, Herwick, and Loevenhart (2) reported that procaine intravenously caused a lowering of blood pressure with absence of vagal stimulation, and that the failure of the heart was the primary cause of death from intravenously administered procaine in dogs under amytal anesthesia.

Lethal dose determinations were also made upon unanesthetized dogs, continuously injected with 2 per cent procaine until death. The injection was kept as constant as possible at 5 cc./min. The symptoms seen were those commonly described by many workers after parenterally administered procaine (6), (7), (8), (9); excitation, increased extensor tone that usually began at the hind legs and traveled anteriorly, running movements, convulsions, and respiratory failure. The apparent lack of balance in these dogs seemed to be merely an effect of increased extensor tonus. The convulsions observed were of a clonic type and were preceded and followed by running movements, the duration of the attack depending upon the dose. With our methods, the mean dosage to produce excitation was 26 ± 6 mgm./kgm., running movements 27 ± 8 mgm./kgm., and convulsions 41.5 ± 9 mgm./kgm. The mean lethal dose was found to be 62.4 ± 14.6 mgm./kgm. ($s.d._m = \pm 2.7$) from injections into 30 dogs. This fatal dose may be somewhat higher than is ordinarily obtained because

of the method of administration, but is, nevertheless, reliable for the comparisons of the remainder of the study where the same technique was used. If any overshooting occurred, it probably was not of great magnitude as indicated by the rapid deaths of each of 9 dogs after injections of doses only slightly larger than those which the dogs were known to tolerate.

Repeated Administration. Suggestion of a tolerance to procaine was observed in the reactions of one unanesthetized dog who was given graded increasing doses of 2 per cent procaine at a rate of 5 cc./min.

TABLE I

Effect of repeated intravenous injections of procaine on the appearance of running movements

DAY	DOSE GIVEN (MGM./KGM.)	MGM./KGM. AT WHICH RUNNING MOVEMENTS APPEARED
23rd	45	38
25	50	39.1
27	50	41.4
29	55	43
32	60	no record
34	65	46
45	70	35
47	75	40
49	75	42
52	80	51
54	85	49
56	90	55
59	95	no record
61	108	57
98	92 (injection until death)	28

about every 2 days. The starting dose was 4 mgm./kgm. and each dose was increased by 5 mgm./kgm. It was found that the dose necessary to produce running movements became larger each succeeding time provided the 2–3 day interval was used, but that when 11 days elapsed between injections, the running movement dose dropped to below the original level. After a total of 21 injections, this dog had running movements at 57 mgm./kgm. and survived 108 mgm./kgm. of procaine (Table I). To study this phenomenon further, 9 additional dogs were subjected to the same procedure. The mean lethal dose for these animals was 61 mgm./kgm. which is well

within the range of the dogs which had had but a single injection of procaine.

The excitatory state in these dogs appeared when 10–15 mgm./kgm. of procaine had been injected as compared with 26 mgm./kgm. for the animals given but a single injection. But, succeeding larger doses induced an excitation which appeared at higher and higher levels, in some cases after a preliminary drop, with an apparent maximum nearly two to three times that of the initial excitatory dose. In a few cases where pupil size was measured, it was seen that during the course of a large injection, the pupils dilated until after the excitatory stage. Just before the onset of convulsions, the pupils began to constrict and remained in the constricted state throughout the period of convulsions. Pupillary dilatation after parenterally administered procaine has been described by MacGregor (10) but no description of the constriction that was observed when larger doses were injected could be found.

Thus it was found impossible to induce tolerance to the lethal effects of intravenous procaine in 9 dogs by the same procedure which had induced an apparent tolerance in one dog. However, with the repeated injection of the drug there occurred an increase in the dose necessary to produce excitation, which might be interpreted as one indication of acquired tolerance. This phenomenon was unexpected in view of the reactions of animals to repeated administration of many other convulsant drugs.

On the other hand, rats which were subjected to a similar procedure showed increasing susceptibility to procaine rather than any tolerance. A series of 33 rats were given graded, increasing, intraperitoneal doses of 2 per cent procaine solution every 2 days, starting with 40 mgm./kgm. and increasing each dose by 20 mgm./kgm. The convulsant dose was 160 mgm./kgm. and all the rats were killed by 200 mgm./kgm. Essentially the same results were found in 15 rats subjected to this procedure daily instead of every 2 days. That these data indicate a much greater susceptibility in the treated animals is shown by the fact that 10 previously uninjected rats under the same environmental conditions showed only a 20 per cent mortality when injected with 200 mgm./kgm., and 30 others gave an 80 per cent mortality from 300 mgm./kgm., the value reported by Rose, Coles, and Thompson (11) to be the median lethal dose.

These experiments on rats were performed in the hot summer months. Sievers and McIntyre (12) have shown that the toxicity of procaine for white mice increases with the temperature, and caution about administration of procaine during the summer. Mice are known to have poor temperature regulation, but to exclude any possibility of the temperature having been a factor in the rats, the entire experiment was repeated in the winter months. That the temperature affects the toxicity in rats as well as mice was shown by the convulsant dose in the rats in the winter group being 200 mgm./kgm. and all being killed by 280 mgm./kgm. Nevertheless, there was still evidence of increased susceptibility from repeated injections since 10 normal rats under the same environmental conditions showed only a 40 per cent mortality at 280 mgm./kgm. In terms of median lethal doses, the treated rats had an M.L.D. of 220 mgm./kgm. as compared to 285 mgm./kgm. for the controls. The above experiments demonstrate that increased susceptibility developed with a progressive increase in the dose administered.

Whether the same phenomenon would result from repeated injections of a fixed dose remained to be determined. Accordingly, daily intraperitoneal injections of 100 mgm./kgm. as 2 per cent procaine were given to 166 rats for various periods of time up to 5 weeks. This dose, which produced no symptoms at first, soon resulted in the appearance of spastic hind legs and running movements. By the end of 5 days of daily injections, the majority of rats showed convulsions, which were present every day. At the end of the injection period, these rats were given 300 mgm./kgm. of procaine intraperitoneally, the mortality being 85 per cent for both these rats and for 30 normal rats from the same colony. It would appear, therefore, that repeated administration of fixed doses leads to a greater susceptibility with respect to convulsions but not to mortality. In this respect, procaine is not unique since other convulsant drugs are known to lead to a greater susceptibility from repeated administration of equal doses.

Circulatory Effects. The fall in blood pressure after intravenous procaine injections and subsequent circulatory failure was reported by Knoefel, Herwick, and Loevenhart (2) to be the primary cause of death in dogs under amytal anesthesia. The mechanism of this fall in blood pressure is uncertain. Eggleston and Hatcher (8) are of the

opinion that this fall in blood pressure is due to a weakening of the heart and not any immediate vasodilatation. By using perfused frog legs and measuring blood outflow from the abdominal veins, Swanson (13) reported that while cocaine produced a constriction in dilutions of 1:1000 and 1:2500 and a dilatation in dilutions of 1:5000, procaine in the same concentrations produced neither a constriction nor a dilatation. To determine the effects of procaine on the peripheral vascular bed, the blood flow through the hind limbs of dogs under pentobarbital anesthesia was measured by a modification of the technique described by Lawson and Holt (14). Instead of the differential manometer used by these workers, small bore mercury manometers were connected to the femoral artery above a constriction and to a small branch below it. With this procedure, injections of vasoconstrictor or vasodilator substances made into the artery above the constriction will cause a change in blood flow which can be measured by changes in the differential of pressure. At the end of the experiments, whenever possible, the constrictions were calibrated by measuring, directly, the flow below the constriction necessary to produce manometer deflections in the ranges observed during the experiments.

In each of 8 dogs, procaine was observed to be a peripheral vasodilator. 0.1–50 mgm. of procaine in 2 per cent solution, injected intra-arterially, caused an increased blood flow through the dog's limb. (*fig. 1*) The response to injections became more variable after a large number of injections had been given so that no quantitative comparisons could be made. Although procaine may cause weakening of the heart as Eggleston and Hatcher (8) believe, the peripheral vasodilatation after procaine administration is a definite factor in the immediate fall in blood pressure.

The circulatory effects of procaine were not great enough to cause death in these as well as the other animals, the circulation being maintained after respiratory movements had ceased.

Respiratory Stimulants as Antidotes. Since the depression of respiration was found to be the primary effect of procaine administration, several respiratory stimulants were tested for their antidotal power. Alpha lobeline, caffeine, benzedrine, and metrazol were administered to both normal and anesthetized dogs simultaneously with the procaine or at the time of respiratory depression or stoppage. The

efficacy of artificial respiration in reviving dogs whose respiration had been stopped by procaine was also tested.

Twelve dogs under paraldehyde intravenously were rapidly injected with 10 mgm./kgm. of procaine in 2 per cent solution to which 100–300 mgm. of metrazol in 10 per cent solution had been added. Seven dogs survived the injection of this mixture. After 20 minutes to an hour, the 7 survivors were injected with the same dose of procaine

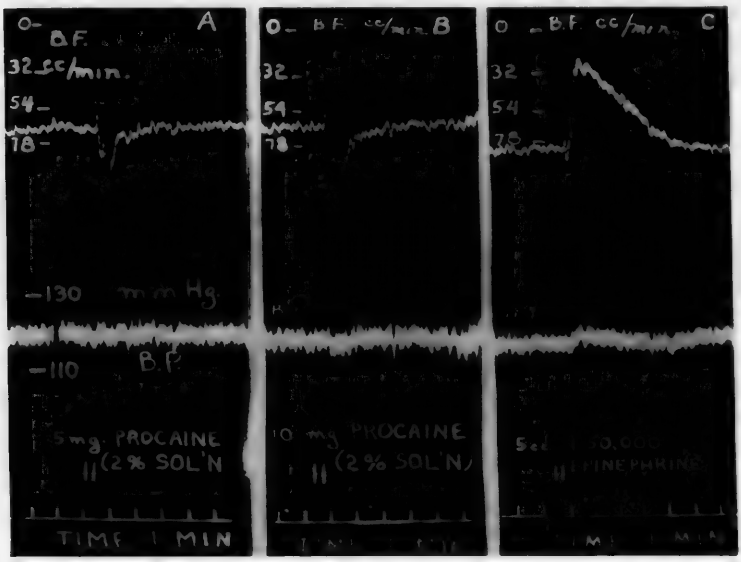

FIG. 1. Effect of intra-arterial injections of procaine and epinephrine on bloodflow through the dog's hind limb. B.F. = blood flow. B.P. = blood pressure.

alone, and 5 died. The 2 remaining dogs were given 15 mgm./kgm. procaine plus metrazol which injections proved to be fatal.

Since metrazol appeared to have some protective action in these dogs the drug was tried on normal unanesthetized dogs which were given 80 mgm./kgm. procaine (as 2 per cent solution) intravenously at a rate of 5 cc./min. To this solution, 100–300 mgm. of metrazol in 10 per cent solution were added. The mean lethal dose, 59 mgm./kgm., fell into the range already determined for procaine alone. By adding the metrazol to the procaine, the mean convulsant dose was

lowered to 19 mgm./kgm. Both metrazol and procaine are convulsant drugs and together they have additive actions. Although no lowering of the convulsant level was observed when only 0.2 mgm./kgm. of metrazol were added to the procaine solutions, there was no evidence of significant antidotal action in each of 6 dogs.

Although one-half of the anesthetized animals responded favorably to metrazol, it was concluded that metrazol could not have any clinically significant protective action against procaine, since it had no effect in counteracting the toxic effects in unanesthetized dogs, and its convulsant property is a definite disadvantage.

No success in reviving dogs was obtained from injections of metrazol caffeine, alpha lobeline, and benzedrine in doses which normally stimulate respiration in the dog, when the injections were made at the time of respiratory depression after lethal doses of intravenously administered procaine. Administration of artificial respiration, immediately after respiratory movements had ceased, was usually successful in reviving dogs. In some cases, the addition of epinephrine intravenously was necessary.

These experiments demonstrate that the respiratory stimulants tested had little value as antidotes against procaine intoxication. Although this work was not undertaken to determine the site of respiratory paralysis in procaine poisoning, the recent work of Bonnycastle (15) who showed that the phrenic nerve roots in the spinal dural sac were paralyzed by concentrations of procaine which the respiratory center in the medulla withstands, also indicates the futility of using central respiratory stimulants as therapeutic agents in procaine intoxication.

SUMMARY AND CONCLUSIONS

1. The mean lethal dose of 2 per cent procaine hydrochloride solution injected intravenously at a rapid rate in 20 dogs anesthetized with paraldehyde, was found to be 9.7 ± 3.8 mgm./kgm. ($s.d._m = \pm.85$).

2. In 30 normal unanesthetized dogs, the mean lethal dose of 2 per cent procaine injected intravenously at a rate of 5 cc./min., was found to be 62.4 ± 14.6 mgm./kgm. ($s.d._m = \pm2.7$).

3. No apparent tolerance to the lethal effects of procaine could be induced in dogs by the repeated intravenous injections of graded

increasing doses of the drug. An indication of a tolerance with respect to the symptoms was obtained.

4. Susceptibility to procaine was increased in rats by repeated intraperitoneal injections of graded increasing doses of the drug. As in mice, procaine toxicity increased with a rise in environmental temperature.

5. Like some of the other convulsant drugs, procaine susceptibility was increased in rats by repeated injections of equal doses of the drug.

6. In dogs under pentobarbital anesthesia, intra-arterial injections of procaine in doses of 0.1–50 mgm. acted as a peripheral vasodilator as measured by blood flow through the hind limb. This vasodilatation is probably a factor in the immediate fall in blood pressure after intravenously administered procaine.

7. In both unanesthetized and anesthetized dogs, moderate doses of metrazol injected intravenously and simultaneously with lethal doses of procaine, were found to have little clinically valuable antidotal action. In dogs anesthetized with paraldehyde, metrazol, caffeine, alpha lobeline, and benzedrine had no effect in restoring respiration after lethal doses of procaine. Prompt administration of artificial respiration immediately after cessation of respiratory movements was usually successful.

The author wishes to express her sincere gratitude to Dr. A. B. Luckhardt for his stimulating suggestions and guidance.

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PENETRATION OF PHENOL IN TOOTH STRUCTURE[1]

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INTRODUCTION

Phenol (carbolic acid) is a hydroxybenzene, C_6H_5OH, first prepared by Runge in 1834 from coal tar, and by Church in pure form in 1871. Today phenol is obtained from coal tar or synthesized from benzene or a pure analine oil. It occurs as colorless needle-shaped crystals, with a characteristic odor and acrid, burning taste, and has a melting point of 40°C. Aqueous solutions contain 5 per cent or 90 per cent phenol; however it is soluble in glycerin, alcohol, fixed and volatile oils, ether and chloroform, and when first dissolved in glycerin or alcohol, phenol is freely miscible in aqueous solutions in any concentration. Since the antiseptic power of phenol depends on the entire molecule, its action as an antiseptic is not reduced when it is mixed with alcohol or glycerin. Although slightly acid to litmus, phenol is more closely related to the alcohols than acids.

One of the more commonly used qualitative tests for the presence of phenol is the bromine water test. This test is accurate to approximately 1 part of phenol in 50,000 parts water. A saturated aqueous solution of bromine converts phenol into crystals of symmetrical tribromophenol. Certain compounds other than phenol, amines for example, also form precipitates with bromine water.

The opinions of different persons regarding the method of application, the mode of action and the effect, as well as indications for and against the use of phenol in cavity sterilization (and desensitization) vary greatly. The method of using phenol most often depends upon previous teaching or clinical results, with its use on an empirical basis.

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For these reasons the present study was undertaken in an effort to determine: 1. The degree of penetration of phenol in tooth structure, as regards its possible self-limiting action due to coagulation of organic matter. 2. The effect of phenol on the dental pulp. 3. Whether on a rational basis one should recommend or condemn the use of phenol in cavity sterilization.

THE LITERATURE

Dental literature in the early 1860's contained articles describing the uses of phenol (carbolic acid) in dentistry. Brophy (*2*), in 1861, compared the chemistry and therapeutic application of phenol and creosote. He stated that while in medicine phenol was definitely preferred, in dentistry, for no good reason, creosote was given preference. Brophy preferred phenol. C. Calvert (*3*), 1864, discussed the therapeutic aspect of phenol.

It was recognized that phenol is a protoplasmic poison producing death of tissue proteins and coagulating albumin. Before many years had passed, the chief interest in the use of phenol was centered about the question of whether or not this drug limited its own penetration in tissues by this coagulation of the protein. Because of the enormous amount of root-canal therapy being done and the use of phenol in the treatment of putrescent pulp canals, this question was of great practical importance. The use of phenol in cavity sterilization received little consideration. A great deal of experimental work was done by 2 men who held opposite views on the question of the self-limiting action of coagulating agents. Harlan (*4*) felt he could show that coagulating agents, including phenol, formed an impenetrable coagulum, thus limiting the extent of their own effectiveness as germicides. York (8) held the opposite opinion and claimed by his many experiments to prove that coagulants did penetrate beyond the visible areas of coagulated albumin.

In 1895, Harlan (*5*) stated that, "A coagulating agent will penetrate albumin, but when it has penetrated to a certain point of satisfaction of the drug, it ceases to give any further diffusion; it stops itself by its own action." He reported the results of experiments which he claimed proved that coagulating agents are self-limiting in their action.

York (*8*), in 1897, presented a number of experiments to show that

phenol will diffuse through dentin in freshly extracted teeth. He also showed the diffusion of phenol through coagulated egg albumin.

Whether the coagulating property of phenol renders it self-limiting in its antiseptic action is still a question. Blayney (1) says, "Some have considered phenol as belonging to the class of self-limiting antiseptics. However, this is not the case, as phenol has greater penetrating powers than any other antiseptic because it does not combine chemically with the tissue, since phenol may be removed from the tissues by certain solvents." McGehee (7), on the other hand, claims, "Phenol is not desirable for treatment of putrescent pulps on account of its lack of penetration and low germicidal powers under the conditions present."

EXPERIMENTAL METHODS

The author's first experiments were concerned with egg albumin, and the results corroborated the statement of York that phenol will penetrate coagulated egg albumin.

The next group of experiments was done on human teeth both in vitro and in vivo. Phenol was placed in 10 pulp cavities and 6 prepared cavities, in vitro, and in 13 prepared cavities in vivo. As a control, 7 cavities were prepared in vivo but not phenolized. The following experiments were performed on teeth in vitro.

Experiment 1. (Repeats experiments done by Harlan and York). The pulp was removed from freshly extracted, sound teeth, either by enlarging the apical foramen and cleaning out the pulp canal and pulp chamber through the enlarged apical foramen, or by opening into the pulp chamber from the occlusal or lingual surface and removing the pulp in this manner. (In those teeth where the pulp had been removed through an occlusal or lingual opening, the apex was carefully sealed with gutta percha and cavity varnish.) The pulp chamber and root canals were dried with cotton points, and 90 per cent phenol, colored with a dye, (acid fuchsin, gentian violet, methylene blue, and eosin Y were tried, and eosin Y was found to be most satisfactory), was injected into the pulp cavity. Care was exercised so as to prevent phenol from flowing over the external surface of the tooth. The pulp cavity was then sealed with gutta percha and cavity varnish. The teeth were washed in running water for one-half hour, then placed in

5 cc. distilled water or 0.9 per cent saline solution. Those teeth where the only opening had been through the apical foramen were suspended crown downwards so that the apical one-third of the root remained out of the solution. This excluded the possibility of phenol leaking through an imperfectly sealed opening.

At 2 hour intervals beginning 14 hours after the teeth were placed in the liquid, 1 cc. of the solution was withdrawn and tested with bromine water for the presence of phenol. The earliest period at which there was a visible precipitate is shown in Table I. As a result of this experiment eosin Y was chosen as the tracing agent for the penetration of phenol, because when the bromine water test first became positive the teeth were sectioned, and the eosin Y had penetrated through the dentin with the phenol, whereas some other dyes had not.

TABLE I

TOOTH	AGE OF PATIENT	PULP REMOVED	DYE	SOLUTION	FIRST PRECIPITATE
$\underline{5}\vert$	39	Through apex	Acid Fuch.	0.9% Sal.	20 hours
$\overline{4}\vert$	45	Through occlusal	Eosin Y	0.9% Sal.	22 hours
$\underline{4}\vert$	49	Through apex	Acid Fuch.	0.9% Sal.	20 hours
?	?	Through apex	Acid Fuch.	Distil. H_2O	20 hours
?	?	Through apex	Acid Fuch.	Distil. H_2O	18 hours
$\lceil\overline{3}$ (Non-vital)	47	Through apex	Eosin Y	Distil. H_2O	22 hours

That phenol was shown to be present in the solution in which the teeth were suspended, in all cases, proves that phenol does pass through the tooth structure.

Experiment 2. The pulps were removed from sound, freshly extracted teeth through an occlusal opening in each tooth. The apical foramen was sealed with gutta percha followed by cavity varnish. The pulp cavity in each tooth was filled with 90 per cent phenol colored with eosin Y. The occlusal opening was carefully sealed with gutta percha and cavity varnish. The teeth were wrapped in gauze moistened with 0.9 per cent saline and placed in an incubator at body temperature. Twenty-two hours later the teeth were sectioned transversely in oil, the sections ground in oil and mounted in Canada balsam. (Eosin Y is soluble in water but not in mineral oil. If the tooth is ground in oil the eosin is not carried over the tooth's surface in such a

way as to make it difficult to determine to what extent the dye had actually penetrated.) Ground sections of teeth used in the above experiment showed the distance the colored phenol had penetrated the dentin in 22 hours. With high-power magnification enamel spindles and tufts stained by the colored phenol were visible.

Experiment 3. A cavity was prepared in each of several freshly extracted sound teeth. The cavity was swabbed with colored 90 per cent phenol and sealed. Each tooth was wrapped in moist gauze and placed in an incubator at body temperature. After a number of hours the teeth were removed from the incubator, sectioned and ground in oil. Table II gives details regarding the method of treatment for each tooth.

TABLE II

TOOTH	PATIENT'S AGE	CAVITY	DYE	HOURS IN INCUBATOR
$\underline{\lfloor 1}$ (Impacted supernumer.)	10 yrs.	Occl.	Acid Fuchsin	20
$\underline{8\rfloor}$	19 yrs.	Occl.	Gent. Violet	24
$\overline{8\rfloor}$	30 yrs.	Buccal	Eosin Y	25
$\overline{\lfloor 3}$	50 yrs.	Labial	Acid Fuchsin	36
$\overline{\lfloor 5}$	50 yrs.	D-O	Acid Fuchsin	43
$\underline{\lfloor 1}$	56 yrs.	Labial	Eosin Y	21

There are marked variations in the permeability of the dentin to colored phenol. The so-called metamorphosed dentin, which appears dark in transmitted light, is relatively impermeable to the phenol, while the "young" dentin is readily permeable. Another striking feature is the manner in which the colored phenol follows the curvature of the dentinal tubuli from the base of the cavity to the pulp. Penetration of the colored phenol from the base of the cavity to the pulp occurred in a 30 year old patient in 25 hours (Table II, case 3). There was some incisal attrition in the first incisor of a 56 year old patient yet the phenol had penetrated from the cavity on the labial surface to the pulp in 21 hours (Table II, case 6). In the maxillary left second incisor from this same patient the pulp chamber and canal were filled with phenol and the tooth sectioned after 120 hours. The metamorphosed areas of dentin were still not penetrated by the phenol.

(b) The following experiment was performed on sound teeth in vivo. A cavity was prepared in each of five teeth (Table III). Each cavity was swabbed with 90 per cent phenol colored with a dye, wiped with cotton, and sealed with gutta percha followed by cavity varnish. After a number of hours the teeth were extracted, sectioned and ground in oil.

TABLE III

TOOTH	AGE OF PATIENT	CAVITY	DYE	HOURS BETWEEN CAVITY PREPARATION AND EXTRACTION OF TOOTH
$\overline{5}$	43 yrs.	Buccal	Gent. Violet	45 hours
$\overline{4}$	43 yrs.	Buccal	Eosin Y	45 hours
$\overline{3}$	48 yrs.	Labial	Eosin Y	23 hours
$\overline{3}$	50 yrs.	Distal (Carious)	Eosin Y	172 hours
$\overline{3}$	50 yrs.	Labial	Eosin Y	20 hours

TABLE IV

TOOTH	AGE OF PATIENT	CAVITY	PHENOL	TIME BETWEEN CAVITY PREPARATION AND EXTRACTION
$\underline{1}$	44	Labial	+	14 days
$\underline{3}$		None	—	
$\overline{3}$	41	Labial	+	72 hours
$\overline{1}$		None	—	
$\overline{5}$		None	—	
$\overline{3}$		Labial	—	
$\overline{1}$		None	—	
$\overline{5}$		None	—	
$\underline{2}$	36	Labial	+	48 hours
$\underline{4}$		Buccal	—	
$\overline{4}$	44	Buccal	+	53 hours
$\overline{3}$		Labial	—	

The tooth in which the eosin Y was used as a tracing agent for the phenol bore a striking resemblance to the teeth treated similarly, but studied in vitro. The carious tooth which was extracted 172 hours after cavity preparation, showed no visible penetration of the colored phenol through the metamorphosed dentin under the carious area. The same type of cavity preparation was carried out on 12 other teeth (Table IV), and the teeth in this group were decalcified for pulp study.

FIG. 1

FIG. 2

FIG. 1. Maxillary right first incisor, 44 year old patient. Cavity (C) was phenolized. Sub-acute reaction area (R.A.) is in that portion of the pulp directly under tubules leading from deepest part of the cavity. D = dentin. O = Odontoblast layer. P = Pulp.

FIG. 2. Higher magnification of area R.A. in *fig. 1*. This zone of reaction consists chiefly of small mononuclear cells. Same legends as *fig. 1*.

Fig. 1 is from the maxillary right first incisor of a 44 year old patient (Table IV). A portion of the cavity is visible, and the tubules from the deepest part of the cavity lead to an area of the pulp where there is an inflammatory reaction, seen better in *fig. 2*. This is a sub-acute reaction, consisting of accumulations of small mononuclear cells. In spite of the damage done to this pulp, there were no subjective symp-

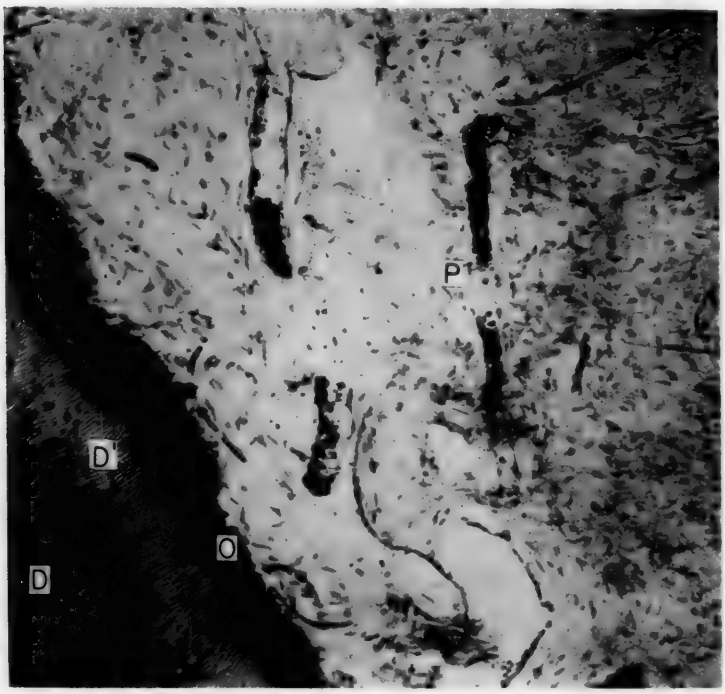

FIG. 3. Maxillary right canine, same patient as *fig. 1*. Normal pulp tissue. No cavity was prepared in this tooth. Legends same as fig. 1. D' = dentinoid.

toms. This reaction is seen 14 days after the cavity was prepared and phenolized. Retrogressive changes, not unusual in the first incisors at 44 years of age, are evident in the odontoblast layer. *Fig. 3* shows the maxillary right canine from the same patient. This serves as a control on the treated tooth and shows normal pulp tissue. *Fig. 4* is from the mandibular left canine of a 41 year old patient. The dentinal

tubules can be traced from the deepest portion of the cavity to the pulp and there is a considerable area of reaction present in that portion of the pulp. This reaction is more acute (3 days) than in the first case.

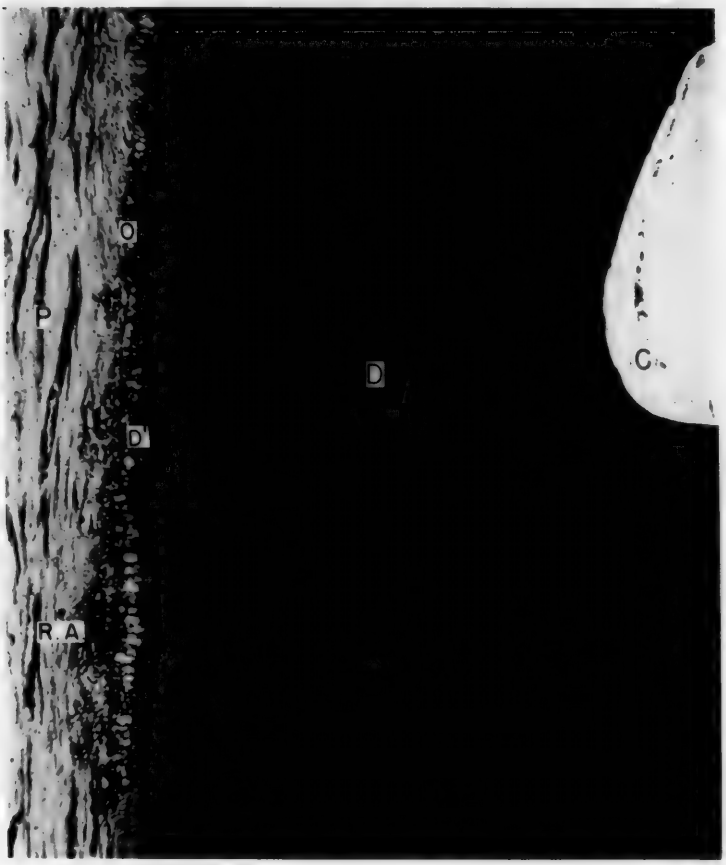

FIG. 4. Mandibular left canine, 41 year old patient. Cavity phenolized. Acute reaction area in pulp under tubules connected with deepest part of cavity. Same legends as *fig. 1.*

Again the separation and retrograde changes in the odontoblasts, which may have occurred as a result of intercellular edema but which is not unusual in a patient of 41 years, is apparent. *Fig. 5* shows the

cavity preparation and pulp tissue in the mandibular right canine in the same patient as in *fig. 4*. This cavity was not phenolized. While this pulp tissue also shows retrogressive changes, there is no area of reaction in the pulp under the cavity.

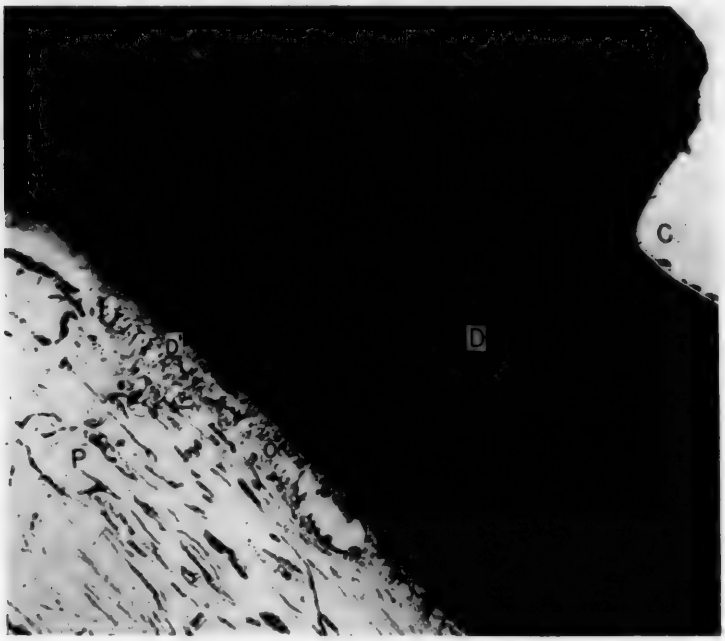

FIG. 5. Mandibular right canine, same patient as *fig. 4*. Cavity not phenolized. No cellular reaction in pulp under cavity. Same legends as *fig. 1*.

DISCUSSION

Metamorphosed dentin is not penetrated by phenol. This indicates that the dentin under carious lesions, as well as secondary dentin, is not so well penetrated, and consequently not so effectively sterilized by phenol as is the permeable "young" dentin in which cavities were prepared in the present experiments. Ninety per cent phenol will certainly coagulate all organic matter which it contacts. However, in "young" dentin it is possible that the dental lymph reduces the concentration of the phenol by dilution to such an extent that by the time the phenol reaches the pulp it does not act as an escharotic, but rather

as an irritant, which may stimulate protective measures on the part of the pulp tissue. The penetration and effect of the phenol therefore depends upon several factors, including: 1. depth of cavity, 2. amount of phenol used, 3. degree of protective metamorphosis of the dentin, 4. amount of secondary dentin present, 5. the condition of the dental pulp (ability to react to irritants).

CONCLUSIONS

Phenol does penetrate tooth structure, and is not self-limiting as a result of its action on organic matter. The degree of penetration depends on several factors. Histologic evidence shows that there are inflammatory reactions in the dental pulp under phenolized cavities even though there are no subjective symptoms. However, such evidence is not sufficient to warrant the condemnation of this drug for cavity sterilization.

SUMMARY

1. The penetration of phenol through tooth structure has been proved by (a) the positive bromine water test and (b) the use of eosin Y as a tracing agent. 2. In vitro experiments showed the penetration from the pulp canal through the dentin and cementum to the surface. 3. In vitro and in vivo tests illustrate that phenol will penetrate from the base of a cavity to the pulp, following the curvature of the dentinal tubules. 4. Metamorphosed dentin is impermeable to phenol. 5. The effect of phenol on the pulp tissue has been shown by means of photomicrographs of experimental and control cases.

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ENAMEL HYPOPLASIA[1]

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In producing enamel hypoplasia experimentally in swine and in rats, Klein (1) has simplified the histological interpretation of these lesions in man. The work of Schour and Smith (2) and more recently that of Diamond and Weinmann (3) has added greatly to our knowledge of this disturbance.

Enamel hypoplasia affects the teeth of human beings in varying degrees of intensity, ranging from an almost microscopic rough surface to deep pits and rills some of which may penetrate to the dentin.

A very severe case of hypoplastic enamel was observed in a girl (C. S.) of 14 years similar to that described by Diamond and Weinmann (3). The enamel surface was not only pitted but all the teeth were of a deep brown color. Because of her unesthetic appearance, when she was 17 years old all of her teeth were removed and replaced by dentures. Investigation was instituted to determine, if possible, the reason for the deep discoloration of all the enamel. The history of the case is meager. The child was born in Liverpool, England and suffered from spinal meningitis at birth. The attending physician is said to have used a serum and the child finally recovered completely. There appears to be no history of rickets or secondary anaemia and it is difficult to determine at this time whether the illness, the serum, or some other factor was the cause of the dental disturbance.

A case similar to this was noted in a boy (R. McC) who had a condition known by the layman as "black teeth." All of his teeth were of a dark brown color and the enamel had the same physical quality as described by Diamond and Weinmann; it could be chipped away with an explorer. The teeth had such a low resistance to the stress of mastication that the mandibular incisors were worn down to the

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gingivae at the age of 11 years. Such cases have been observed occasionally and the teeth have been described erroneously as having no enamel. The reason for this statement is two-fold: 1. the enamel is not differentiated in color from the brown dentin and 2. the enamel is soon worn away due to hypocalcification from all areas which are subject to attrition. No detailed study of this case (R. McC) could be made as no teeth were extracted; also no history was available as the boy was an orphan. The investigation of the other case (C. S.) follows.

FIG. 1. Ground section of premolar with hypoplastic enamel lesions (H). Chronic caries (C) and protective metamorphosis of dentin (MD).

METHOD

Four to 7 ground sections were made of selected teeth. Some were cleared in cedar oil, mounted in Canada balsam or glycerine jelly for permanent preservation. Others were treated with the Cape-Kitchin modification of the celloidin decalcifying method (4, 5) or decalcified under the cover glass with an aqueous, 2 per cent solution of nitric acid.

RESULTS

Figs. 1 and *2* are ground sections of a maxillary premolar and a canine cleared in cedar oil and mounted in Canada balsam. The hy-

poplastic lesions are present in great numbers in both these teeth although they are not clearly visible due to the opacity of the enamel. The appearance of the ground sections of these teeth suggested that the amount of enamel matrix might be abnormally high, and decalcification of some of the sections determined the correctness of this view.

Fig. 3 shows the partially decalcified enamel of the labial plate of a first incisor. The funnel shaped hypoplastic lesions have survived the

FIGS. 2-4

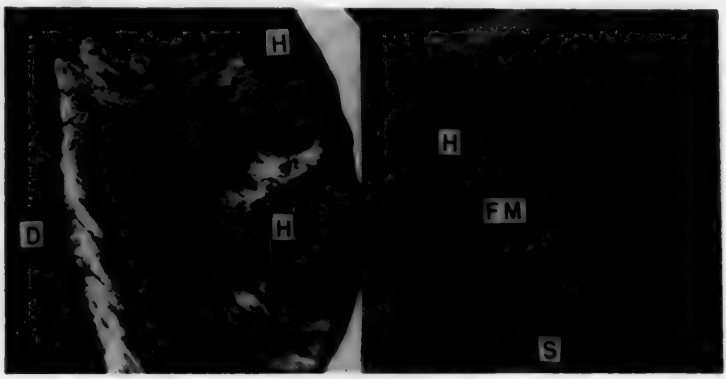
FIG. 2. Ground section of canine with enamel hypoplasia

FIG. 3. Decalcified hypoplastic enamel from canine tooth. D = dentin

FIG. 4. Higher magnification of 4 decalcified hypoplastic lesions (H) showing excessive amounts of organic matrix (M); dentin (D).

action of the acid and are shown to be continuous with the enamel cuticle. Also a greater density of the matrix is noted in the immediate vicinity of the lesions (*fig. 4*) under higher magnification. The enamel which is not affected by the hypoplastic lesions appears in ground section to be quite normal under low magnification. Higher magnification, however, shows that the enamel rods are markedly abnormal in certain areas suggesting that the matrix was mal-formed. The rods

appear coarser and do not follow the usual, slightly wavy course, common to gnarled enamel (*fig. 5*). Decalcification of such areas discloses a fibrous-like matrix (*fig. 6*). Certain of the fibers of the matrix suggest a tube-like structure but this appearance could not be ascertained definitely because the specimen, having been decalcified under the cover glass, could not be viewed under high magnification. The examination of the decalcified sections shows that the amount of enamel matrix of these hypoplastic teeth is abnormally high.



FIGS. 5 AND 6

FIG. 5. Ground section showing abnormal course of enamel rods (R) and spindles (S) in canine (*fig. 2*). H = hypoplasia; D = dentin.

FIG. 6. Coarse fibrous matrix (FM) in decalcified enamel. H = hypoplasia; S = surface of enamel showing excessive amounts of matrix.

DISCUSSION

The works of Klein (1) and Schour and Smith (2) show conditions similar to those noted in this case. The animals Klein used in the experimental production of enamel hypoplasia were swine and rats fed pre- and post-natally on a low calcium high phosphorus diet, while Schour and Smith used excess fluorine to produce hypoplasia. The resemblance of the lesions in *fig. 3* and those produced in the experimental animals is striking each having the same wavy surface of the enamel and similar deep funnel-like depressions.

The deep discoloration of all of the teeth of the patient under consideration does not appear to be related directly to hypoplasia. There

seems to be another factor which prevented the complete calcification of the enamel matrix. Diamond and Weinmann present a new view of this subject. They believe that the enamel matrix contains its normal quota of mineral salts in colloidal form but that these have not crystallized. This hypothesis, however, requires substantiation by a physical chemist. Clinically, this insufficient calcification increases the permeability of such abnormal enamel, enabling the saliva to penetrate it. Saliva carries organic material into the enamel which on decomposition, causes the discoloration of the tooth. This observation is borne out by the in vivo experiments of Lefkowitz (6) who showed that *normal* enamel is impermeable to saliva. Penetration of the dye occurred only in partially decalcified areas, i.e., those zones of the enamel which have become permeable. The clinical observation that incipient carious lesions (partly decalcified enamel) are at first white and later brown suggests that this discoloration is due to the penetration of the saliva and that only impermeable enamel retains its normal luster. The belief that the discoloration of incipient carious lesions in enamel become discolored merely as a result of the action of light is untrue, as shown by the common observation that these are noted often on the maxillary posterior teeth which receive no illumination whatever.

Hypoplasia of the enamel does not always result in its later discoloration. Many mild forms are noted clinically in which the surface of the enamel is pitted or rilled, but the adjoining normal enamel, covering the remaining parts of the teeth, has its usual glistening appearance, hence the question arises whether discoloration of the enamel is related always to hypoplasia.

CONCLUSION

The present investigation bears out the work of Diamond and Weinmann by presenting evidence that 2 pathological conditions appeared to exist in the teeth under examination, i.e., 1. enamel hypoplasia resulting in localized deficiencies of structure and 2. a disturbance in calcification affecting the entire enamel. The latter condition explains the excessive amount of enamel matrix which caused a pathological permeability from the surface resulting in the intensive discoloration of the teeth.

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ESTIMATION OF SALIVARY PHOSPHATASE[1]

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The close relationship between bone formation and phosphatase was first indicated by Robison (1) in 1923. Since then he and his coworkers have shed much light on the problem of ossification and decalcification in the human body. They have shown that the enzyme phosphatase is one of the prime factors in controlling these processes. Phosphatases occur in most mammalian tissues and their importance and distribution has been reviewed by Folley and Kay (2). Their presence in saliva was demonstrated by Demuth (3). The methods for their estimation in saliva in use at present require too much time, often as long as 24 hours (4). For this reason the following rapid method is proposed.

Native saliva is a mechanical mixture consisting of microorganisms, epithelial cells, food particles, calculi and a more or less viscid opalescent fluid. For an accurate analysis of this mixture it should be centrifuged and the centrifugate and precipitate analysed separately. Some workers have reported that centrifuged saliva contains no phosphatase (5), others are of the opinion that the mouth organisms are largely responsible for salivary phosphatase (6). Many have attempted to find a relationship between salivary phosphatase and dental caries. As an aid in arriving at a definite conclusion on any of these questions a rapid accurate method for the quantitative determination of salivary phosphatase would be valuable.

The usual method for the determination of phosphatase is to hydrolyse a phosphoric ester, such as sodium glycerophosphate, by means of the phosphatase in the saliva and measure the inorganic phosphate liberated after 24 hours at 37°C. In the method proposed a phenolic ester of phosphoric acid, disodium phenyl phosphate, is used as sug-

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gested by King (7) and the phenol liberated measured by means of the Folin and Ciocalteu reagent (8).

EXPERIMENTAL

The samples of saliva, 4 to 5 ml., used for analysis were collected without stimulation, by expectoration into test tubes. It was stored at zero temperature for one, or in some instances two, days. The saliva was then transferred to a 15 ml. graduated conical centrifuge tube and centrifuged for 5 minutes at 2000 r.p.m. The volumes of centrifugate and precipitate were recorded. The centrifugate was then decanted into another tube and the precipitate suspended in sufficient water to make a total volume of 5 ml. For each saliva analysed 6 tubes containing the following solutions were prepared.

TABLE I

TUBE NOS.	BUFFER, ml.	SUBSTRATE, ml.	ENZYME IN ML.	
			Precipitate	Centrifugate
1 and 2	7	2	—	1
3 (control)	7	2	—	1
4 and 5	7	2	1	—
6 (control)	7	2	1	—

Before the addition of the enzyme 5 ml. of dilute Folin and Ciocalteu reagent was added to Tubes 3 and 6. Buffer: Michaelis (9) acetate-veronal at pH 4.0. Substrate: One-twentieth molar disodium phenyl phosphate. Enzyme: For centrifugates 1 ml. of the natural centrifuged saliva and for the precipitates 1 ml. of the aqueous suspension was used.

All the tubes were rocked in a water bath at 37°C. for 1 hour in L-shaped rocker tubes as described by Eastcott (10). 5 ml. of dilute Folin and Ciocalteu reagent was then added to tubes 1, 2, 4 and 5. The contents of each tube were filtered through #32 Whatman filter paper and 10 ml. of the filtrates transferred to 15 ml. volumetric flasks. To each flask 2.5 ml. of 20 per cent sodium carbonate solution was added, the tubes made to volume and the colors compared with a Duboscq colorimeter, after 20 minutes, with a standard phenol solution. The results are calculated on the basis of mg. of phenol liberated per 1 ml. of centrifugate and per 0.2 ml. of precipitate. In the

TABLE II

CASE NO.	VOLUMES IN ML.		PHOSPHATASE UNITS—MG. PHENOL				DEGREE OF SUSCEPTIBILITY TO CARIES
	Centrifugate	Precipitate	Centrifugate per ml.			Precipitate per 0.2 ml.	
			Test	Contr.	Diff.		
24	6.3	0.12	0.151	0.047	0.104	0.510	+++
25	6.0	0.03	0.133	0.048	0.085	0.400	+
30	4.6	0.20	0.133	0.040	0.093	0.325	+++
29	7.3	0.10	0.122	0.049	0.073	0.244	++
21	5.7	0.20	0.243	0.042	0.201	0.228	+++
3	5.1	0.10	0.155	0.054	0.101	0.228	++++
6	5.7	0.09	0.142	0.050	0.092	0.220	+
5	6.0	0.20	0.181	0.038	0.143	0.203	+++
12	5.3	0.28	0.178	0.068	0.110	0.203	+++
8	7.5	0.50	0.185	0.057	0.128	0.202	+++
1	5.5	0.15	0.144	0.045	0.099	0.197	+
35	5.0	0.08	0.144	0.048	0.096	0.192	+++
17	7.8	0.12	0.161	0.069	0.092	0.192	+++
36	4.7	0.28	0.237	0.068	0.169	0.187	+++
4	3.9	0.07	0.180	0.054	0.126	0.186	+++
22	6.1	0.37	0.207	0.058	0.149	0.172	+++
19	8.8	0.32	0.095	0.045	0.050	0.171	+++
26	4.8	0.22	0.119	0.067	0.052	0.170	++
39	4.9	0.30	0.241	0.054	0.187	0.168	++++
11	5.7	0.20	0.089	0.059	0.030	0.166	+++
32	6.7	0.30	0.388	0.057	0.331	0.165	+
10	5.3	0.08	0.073	0.052	0.021	0.160	+
15	6.8	0.23	0.087	0.046	0.041	0.151	+++
7	7.0	0.30	0.206	0.052	0.154	0.143	no report
28	6.2	0.32	0.168	0.056	0.112	0.142	++
20	8.2	0.36	0.091	0.040	0.051	0.140	++++
27	6.8	0.18	0.143	0.060	0.083	0.138	+++
23	6.3	0.12	0.069	0.050	0.019	0.136	+++
13	5.3	0.10	0.069	0.052	0.017	0.130	++
16	7.2	0.25	0.172	0.068	0.104	0.126	++++
38	6.3	0.05	0.071	0.048	0.023	0.124	++
31	5.1	0.21	0.113	0.048	0.065	0.115	+++
14	9.0	0.29	0.114	0.070	0.044	0.110	+++
37	5.6	0.14	0.090	0.044	0.046	0.109	+++
33	7.1	0.30	0.147	0.053	0.094	0.108	+
9	5.3	0.25	0.120	0.062	0.058	0.084	++
2	5.0	0.30	0.118	0.064	0.054	0.082	++
18	7.0	0.15	0.075	0.050	0.025	0.076	+++
34	5.7	0.30	0.128	0.053	0.075	0.067	++

control tubes in the case of the precipitates no blue color or a slight trace only was apparent so these were omitted from the following table. A group or representative results is shown in Table II. The degree of susceptibility to caries is indicated by +(relatively non-susceptible) to ++++ (extremely susceptible).

CONCLUSIONS

A rapid accurate quantitative method for the determination of salivary phosphatase is described. There is no apparent relationship between the phosphatase content of the saliva (either centrifugate or precipitate) and the susceptibility to dental caries in the 39 boys of approximately the same age group who were examined.

My thanks are due to Dr. R. G. Ellis for collecting the salivas and for his critical study of the case histories.

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OBSERVATIONS ON INDUCED DENTAL CARIES IN RATS

II. The Effect of Subcutaneous Injection of Fluoride

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Epidemiological evidence (1), (2), (3), and experimental studies in rats (4), (5), (6), have shown that the process or processes involved in the production of dental caries can be altered by the presence of fluorine in the drinking water or in the food. It has been suggested that the fluoride ion acts either as an anti-enzymatic agent or produces a more resistant tooth substance; whether this action is local, systemic, or both, is not known.

Dean, Jay, Arnold, McClure and Elvove (2) have pointed out that the epidemiological evidence cannot be adequately explained on the presence or absence of dental fluorosis; however, at Bauxite, Arkansas, mottled teeth resisted dental caries attack even though the population had been using a fluoride-free drinking water for the past twelve years (7). Cheyne has interpreted his results (8) to show that the action of fluorine on dental caries in rats was not dependent on the presence of saliva. McClure (9) has shown that the caries resistance of rats is correlated with the amount of fluorine in the diet; also the fluorine content of the molar teeth of these rats changes in direct proportion to the amount of fluorine in the diet. None of this evidence permits conclusions to be made as to whether the action of fluorine on dental caries is local, systemic, or associated with the tooth structure.

The present experiment was planned to eliminate the local action of the fluorine so far as it is related to the presence of this element in the food or drinking water. To this end, the fluorine was administered by subcutaneous injections of NaF, and the dental caries experience of the injected rats was compared with that of a control group and a group receiving 10 ppm. F in drinking water.

[1] From the Division of Infectious Diseases, with the cooperation of the Division of Chemistry and Division of Chemotherapy, National Institute of Health.

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EXPERIMENTAL METHOD

The rats used in this experiment were separated from their mothers at 21–23 days of age and kept on a non-caries producing stock ration and distilled water until they had reached more than 30[2] days of age. After this period litter mates were distributed among 3 groups, i.e., control, injection, and fluoride water. All rats were given food and water ad libitum. The food used for all rats was a caries-producing

TABLE I

Summary of results showing the effect of injected fluoride and water containing 10 ppm. F as NaF on induced caries in rats

TREATMENT	NO. OF RATS	NO. OF LITTERS	RATS WITH CARIES	CARIOUS TEETH			AREAS AFFECTED			TOTAL SCORE	SCORE PER CARIOUS RAT
				Upper	Lower	Total	Upper	Lower	Total		
Controls (distilled water)											
No.	23	21	23	7	70	77	7	145	152	361	—
Per cent	—	—	100	5.1	50.7	27.9	0.03	26.3	19.4	—	—
Ave. rat	—	—	—	0.3	3.0	3.3	0.3	6.3	6.6	15.7	15.7
Injection (dist. water and inject. of fluoride)											
No.	24	21	20	11	53	64	15	138	153	388	—
Per cent	—	—	83	7.6	36.8	22.2	0.06	24.0	18.8	—	—
Ave. rat	—	—	—	0.5	2.2	2.7	0.6	5.8	6.4	16.2	19.4
Fluoride water (10 ppm. F in water)											
No.	20	19	16	3	50	53	3	110	113	279	—
Per cent	—	—	80	2.5	41.7	22.1	0.02	22.9	16.6	—	—
Ave. rat	—	—	—	0.2	2.5	2.7	0.2	5.5	5.7	13.9	17.4

corn meal ration consisting of 66 per cent granulated corn meal (approximately 60 per cent of this meal was retained by a 40 mesh sieve), 30 per cent whole milk powder, 3 per cent alfalfa meal (all of which passed a 60 mesh sieve) and 1 per cent sodium chloride. The control group, and the injection group received distilled water; the third group

[2] The average age of each group when placed on the experiment was: control—32.8 days, 10 ppm. group—32.7 days, injection group—41.3 days.

received drinking water which contained 10 ppm. F as NaF. The injection group received subcutaneous injections of 1 cc. of a solution of

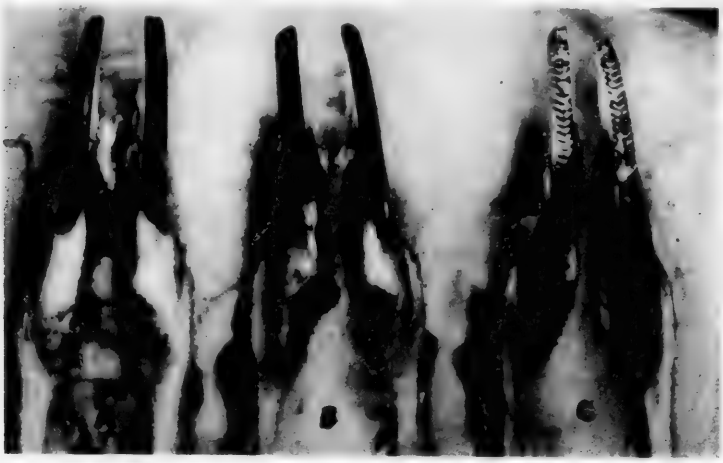

FIG. 1. Lower incisor teeth of rats; A.—control, B.—10 p.p.m. F. as NaF., C.—Injection of 0.5 mg. F. as NaF.

TABLE II

Results of determinations of the ash and fluorine content of the crowns of rat incisor and molar teeth

TREATMENT	NO. OF RATS	INCISORS				MOLARS			
		Enamel		Dentine		Enamel		Dentine	
		% Ash	% F	% Ash	% F	% Ash	% F	% Ash	% F
Controls (distilled water)	23	95.69	.0036	79.45	.0046	95.18	.0034	79.03	.0089
Fluoride water, 10 ppm. in water	20	95.01	.0159	79.01	.0494	95.41	.0128	79.38	.0414
Injection distilled water plus injection of fluoride	24	95.49	.0418	79.44	.1301	95.20	.0131	79.13	.0677

NaF equal to 0.5 mg. F every second day for the first 24 days and daily thereafter, making a total of 86 injections for each rat.

The molar teeth were examined under a low power microscope (magnification × 22.5), the visible cavities being recorded and scored on a

chart similar to the method described and illustrated by Cox, Dodds, Dixon and Matuschak (10). Only the cavities as seen in unsectioned teeth were recorded, grouping male and female results, because sex differences did not warrant separate classification. These results are shown in Table I.

The extent of absorption of fluorine may be judged by observing the fluorine content of the incisor teeth and by noting the degree of dental fluorosis present. Photographs of the incisor teeth, representative of each group, are shown in *fig. 1*.

The crowns of the molar and incisor teeth of the upper and lower right jaws were removed and pulverized to pass through a 60-mesh sieve. The enamel and dentin were separated, using the method described by Manly and Hodge (11). The fluorine content of these enamel and dentin samples was determined by distillation from perchloric acid and titration with thorium nitrate. Details of the procedure followed may be found in an article by McClure (12). The results of these determinations are shown in Table II.

DISCUSSION

The results as presented in Tables I and II seem to indicate 2 major findings, both of which require further discussion.

First: Fluorine, when injected subcutaneously in these rats, did not increase their resistance to induced dental caries. According to the data as obtained, the injected group of rats had as much dental caries as either the control or the 10 ppm. fluoride water group. This negative effect of the injected fluoride was accompanied by an average daily intake of approximately 3 mg. of injected fluorine per kilogram of body weight. This amount approximates the average daily fluorine intake of rats whose drinking water contains 40–50 ppm. F.[3] Judging from the quantity of fluorine retained by the incisor teeth as indicated by the degree of dental fluorosis and by quantitative analyses, a greater per cent of fluorine was absorbed from injection of sodium fluoride than from similar quantities of sodium fluoride given in food or water. According to the results obtained by McClure (9), drinking water containing 40–50 ppm. F should produce a significant reduction in the

[3] These calculations are based on an assumed average daily intake of 10 cc. water. This assumption is based on the results reported by McClure (12).

amount of induced dental caries, however, the results on the injected rats indicate no such reduction. Attention is called to the fact that the group of rats receiving water containing 10 ppm. F show little, if any, significant reduction in dental caries. Whether this is due to the age of the rats, or whether 10 ppm. F represents a borderline amount with respect to induced rat caries as is shown by McClure's results is not known (9).

The above result with respect to injected fluoride indicates that the action of fluorine in reducing dental caries in rats is possibly more closely related to local or oral factors than to some systemic influence. This finding makes it appear improbable that a significant reduction of induced rat caries by fluorine comes through the saliva; a finding which is in accordance with the results reported by Cheyne (8) for rats with extirpated salivary glands.

Second: The results as shown in Table II indicate that fluorine, when injected subcutaneously in rats, increased the amount of fluorine in the enamel and dentin of both the incisor and molar teeth. This increase is related to the fluorine intake of the rat since the teeth of the fluoride water group also show a fluorine increase but the increase was not as great as was found in the teeth of the injected rats. It may also be noted that the quantity of fluorine absorbed in the dentin of both the incisor and molar teeth was greater than in the enamel. These results based on fluorine analyses permit the assumption that the composition of the enamel and dentin of molar teeth of rats may be altered by way of the blood stream through internal surface phenomena even after the crowns of these teeth have erupted in place.[4]

The above statement must be qualified because of the possibility that some, if not all, of the extra fluorine deposited in the enamel may have come from the saliva. Considering the low results obtained by McClure (13) as to the amount of fluorine in human saliva, and the fact that according to Wills (14) the time during which radio-active fluorine was present in the saliva of injected cats was very short, it appears unlikely that the observed increases of fluorine in the enamel of the injected rats were due to an adsorption of this element from the saliva. These observed results pertaining to the injected fluoride and

[4] According to Addison and Appleton (18) and Mellanby (19) all the molars would be erupted at approximately 35 days.

its effect on enamel fluorine make it seem doubtful that an increase in the fluorine content of the enamel of rats given a fluoride drinking water comes entirely through the surface as Perry and Armstrong (15) have recorded.

According to the data presented in Tables I and II, it appears that even though the fluorine content of the molar teeth of rats was increased, there was no proportionate increase in the resistance of these teeth to dental caries. This finding may not be in contradiction to Cox, Matuschak, Dixon, Dodds and Walker (16) and Armstrong and Breckus (17) whose results suggest that optimum amounts of fluorine in the teeth make for increased resistance to caries. In other words, fluorine in this case has been added following eruption and its action against caries may not be similar to the action of fluorine which has been added during the formative period of the tooth.

In general, the results for injected fluorides thus far obtained warrant the assumption that the action of fluorine on induced dental caries in rats is associated with local factors, principally those factors associated with the diet of the rat.

SUMMARY

Subcutaneous injection of sodium fluoride produced no significant reduction in induced dental caries of rats.

The fluorine content of the enamel and dentin of the incisor and erupted molar teeth of rats was increased both by subcutaneous injection of sodium fluoride and by feeding water containing 10 ppm. F.

The observations on the injected rats would seem to rule out any systemic influence of fluorine on induced rat caries when the agent (F) is introduced during the post eruptive tooth period.

The results of this experiment indicate that although the fluorine content of the molar teeth of rats injected with sodium fluoride was increased, these teeth did not show increased resistance to caries.

Acknowledgment is made to Dental Surgeon H. T. Dean for advice and suggestions used in this experiment.

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DISTRIBUTION OF SODIUM IN THE TEETH AND BONES OF DOGS AS SHOWN BY THE RADIOACTIVE ISOTOPE[1]

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Although 25 per cent of the sodium in the body is concentrated in the skeletal tissues (1), a survey of the literature shows that few chemical analyses of teeth and bones for this element have been reported. This may be a result of the difficulty usually associated with sodium micro-chemical methods. The figures given by Gabriel (2), Logan (3), Harrison (4), and Bowes and Murray (5), shown in Table I, are in fairly good agreement. It is of interest that the values given for enamel are, in general, considerably higher than those for dentin and are comparable to those for bone.

Based on the analyses of the tissues and the interstitial fluid of whole animals, Harrison, Darrow and Yannet (1), were unable to account for all of the body sodium. The ratios of Na : Cl in the skin, muscles, and other viscera, excepting the liver and kidneys, were identical with the calculated ratios for interstitial fluid, while those for the entire animal were always higher. Except for a small amount in the liver, this "excess" sodium was in the skeletal system, but whether it is a part of the organic matrix or the mineral phase of bone is not known. If the "excess" sodium which is in bone is credited to the cells, the concentration of Na plus K in the cells greatly exceeds that of the interstitial fluid. Peters (6) believes this to be theoretically impossible and suggests that some of the Na or K, or both, is in an inactive form. It was hoped that an experimental study designed to indicate the mechanism by which sodium is utilized by the teeth and supporting bony structures, might furnish some evidence on this particular point.

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METHODS

The radioactive sodium was prepared by the bombarding of sodium metal with deuterons in the cyclotron.[2] The nuclear reaction is as follows:

$$_{11}Na^{23} + {}_{1}H^{2} \longrightarrow {}_{11}Na^{24} + {}_{1}H^{1}$$

The unstable isotope $_{11}Na^{24}$, has a half-life of 14.8 hours.

The radioactive samples were diluted to volumes of 10 cc. with a 0.1 N NaCl solution. A 0.5 cc. aliquot of each sample was made up to a volume of 1 litre and used in the determination of the standard

TABLE I

TISSUE	GABRIEL* (PER CENT)	LOGAN** (PER CENT)		HARRISON (PER CENT)	HOWES AND MURRAY (PER CENT)
Human bone	0.77		—	—	—
Dog bone	—		0.48	0.43	—
Monkey bone	—		—	0.65	—
Human enamel	—	young	0.71	0.68	0.25
		old	0.90		
Human dentin	—	young	0.31	—	0.19
		old	0.30		
Dog enamel	—		0.56	—	—
Dog dentin	—		0.30	—	—
Ox enamel	0.82		—	—	—
Ox dentin	0.59		—	—	—

* Calculated from Na_2O

** Calculated from Na + K.

decay curve. Four adult dogs and 1 young dog (6–8 months) were given intravenous injections of 9.5 cc. of the radioactive material, and 12 hours later the animals were sacrificed. Representative dental and bony tissue were sampled for analysis. Bone from the maxilla, mandible and usually the femur shaft were removed from each animal, cleaned and dried, and broken up in a diamond mortar.

A similar procedure was used with the whole teeth samples from each experimental animal. These consisted of a canine, incisor, pre-

[2] The radioactive sodium was prepared in the physics department of the University by Dr. S. N. VanVoorhis, and was made possible through the generosity of the Rockefeller Foundation.

molar and molar. In addition, 2 deciduous canines were included in the experiment with the youngest animal (dog No. 4). The remaining teeth from each animal were dried and powdered and divided into 2 parts. One portion was used for enamel and dentin determinations. These tissues were separated quantitatively by the method of Manly and Hodge (7). The second portion had the organic matrix removed by glycol-ash extraction (8).

In dog 3 an attempt was made to determine the rate of deposition of the radioactive sodium in the dentition. This animal was anesthetized and the crowns of 1 canine and the second incisor were removed at 3, 6, 9 and 12 hours following injection and treated in a manner similar to that for whole teeth.

Aliquots were taken of all the tissues, the preparation of which has been described. These were dissolved in 6N HCl and the radioactivity determined by the Geiger-Müller scale-of-four counter. At suitable intervals the radioactivity of the standard radioactive sodium solution was determined as well as the background count and the counter sensitivity. From these data the Na^{24} distribution was calculated in the accepted manner.

RESULTS

A considerable variation existed in the total amount of radiosodium deposited in the dental and skeletal tissues of the various dogs. However, the ratios of Na^{24} uptake by comparable tissues themselves were fairly constant in each animal (Table II).

The values for the maxilla and mandible are of the same order as those for the dense bone from the femur shaft. The average value for the maxilla (in percentage of total dose per gram of tissue, times 10^{-3}) was 14.7, that for the mandible 14.8. The femur gave an average value of 17.8.

No significant variation could be found between the 4 types of whole teeth examined. However, the deciduous canines in the young animal showed the highest values observed in the whole series. The permanent canines in this dog were incompletely calcified, yet gave no higher values than the other, completely calcified teeth. The average value for the incisors was 10.1, for the canines 9.2, for the premolars and molars 11.2, and 10.4 respectively, and for the deciduous canines 31.7.

The average value of 5.6 for the enamel was approximately one-half that for the dentin, 11.0. The dentin value, as would be expected, is comparable to that for whole teeth as it makes up the greater percentage of the whole tooth.

The values for tooth substance from which the organic material had been extracted, were very low and gave an average of 1.4. This indicates a removal of 87 per cent of the radiosodium.

TABLE II

Per cent of total dose per gram of tissue ($\times 10^{-6}$)

TISSUES	DOG 1	DOG 2	DOG 3	DOG 4*	DOG 5	AVERAGES
Incisor	7.9	1.8	3.8	24.5	12.8	10.1
Canine	6.3	1.4	2.7	21.4	14.6	9.2
Premolar	12.1	1.8	3.7	23.5	15.2	11.2
Molar	6.8	2.5	3.8	25.5	13.4	10.4
Maxilla	13.4	4.4	5.7	27.5	22.8	14.7
Mandible	12.6	4.0	5.1	27.7	23.0	14.8
Enamel	2.8	0.8	1.2	12.2	11.0	5.6
Dentin	8.4	2.8	2.4	28.4	13.2	11.0
Glycol ash extr.	1.4	0.5	0.5	3.2	1.6	1.4
Femur shaft	—	4.1	—	25.5	24.3	17.8
Deciduous canine	—	—	—	31.7	—	31.7

* 6–8 months of age.

TABLE III

Per cent of total dose per gram of tissue ($\times 10^{-6}$)

TISSUE	3 HRS.	6 HRS.	9 HRS.	12 HRS.
Incisor	0.49	0.69	0.97	2.10
Canine	0.64	0.70	1.04	1.45

With dog 3 it was found that a maximum radiosodium accumulation in the incisor and canine was not reached before approximately 12 hours (Table III).

DISCUSSION

The variations observed in weights and ages of the different animals were probably responsible in part for the differences in their Na^{24} uptake. The first 3 dogs averaged 17 kg. and appeared to be some-

what older. Dogs 4 and 5 averaged about 9 kg. and were definitely younger.

The fact that slight variations could be found in the Na^{24} uptake by individual whole teeth indicates that there may be some correlation between the amount of radiosodium uptake and the size of the pulp chamber. The canines, which had the smallest average uptake, on gross examination, seemed to have proportionately the smallest pulp chamber, and the premolars, which had the greatest values, the largest pulp chamber.

The values for enamel and dentin are of interest in that dog dentin, having a relative sodium content of only about one-half that of the enamel (3), shows a Na^{24} uptake almost double that of enamel. This might be expected because of the close proximity of the dentin to the pulp, with its adequate complement of body fluids.

The greatly decreased values for the glycol-ash extracted tooth tissue seems to indicate that the majority of the sodium in calcified tissues which undergoes active exchange is found in the organic matrix. Some of the radiosodium not removed in the glycol extraction might possibly have been readsorbed by the tricalcium phosphate. Preliminary studies support this possibility.

Volker and Sognnaes (9), working with radiophosphorus, have found that the extraction of the organic matrix from teeth resulted in the removal of only about 5 per cent of the labeled element. Obviously, sodium presents an entirely different problem and only further studies will reveal the complete nature of its existence in calcified tissue.

Our findings on the rate of deposition of Na^{24} in teeth are in accord with those of Manery and Bale (10) for bone, who found that the maximum accumulation was reached in slightly less than 12 hours.

SUMMARY

Radioactive sodium was administered intravenously to 4 adult dogs and one young dog. Considerable variation was found to exist between the total Na^{24} uptake of different animals but the ratios of radiosodium uptake by the various tissues within individual dogs, were fairly constant.

The Na^{24} uptake by the maxilla, mandible, and femur were of a similar magnitude. No significant variation could be found in the radio-

sodium distribution of whole teeth from the same animal. The dentin gave values approximately twice that of enamel. Extraction of the organic part of whole tooth substance resulted in removal of 87 per cent of the labeled element. The maximum deposition of Na^{24} did not take place in teeth until almost 12 hours following injection.

The authors wish to thank Drs. H. C. Hodge, J. F. Volker and N. L. Kaltreider for their interest and assistance in this work.

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THE DISTRIBUTION OF RADIOACTIVE PHOSPHORUS IN THE TEETH OF EXPERIMENTAL ANIMALS[1]

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The dental hard tissues have the highest inorganic to organic ratio observed anywhere in the body. The numerous chemical analyses of the inorganic fractions of the teeth found in the literature are in fairly good agreement. In general, the enamel has approximately 1 to 2 per cent organic material and the principal mineral constituents are calcium and phosphorus which make up 36 and 17 per cent of the dry weight, respectively. The dentin contains approximately 25 per cent organic material and of the total dry weight 26 per cent is calcium and 12.5 per cent phosphorus. Unfortunately, this constancy has been interpreted as indicating that the fully formed tooth is not subject to metabolic changes.

The use of labeled isotopes with which the metabolism of a single dose of ingested substance may be traced, offers a suitable method for studying the inorganic metabolism of the teeth. Since the radioactive isotope of calcium, the largest single mineral constituent of the teeth, has certain properties that make it difficult to work with, the more suitable radioactive isotope P^{32} was selected for this study. Besides being the second largest mineral constituent of the calcified tissue, experimental evidence indicates that phosphorus is readily adsorbed by the hydroxylapatite crystal, which is probably the principal molecular component of bones and teeth (1).

Although the dental hard tissues and their supporting alveolar structure constitute only a small portion of the total skeleton, they are the site of 2 as yet unsolved important diseases, dental caries and periodontosis (periodontoclasia). Since it has been suggested that

[1] This work was supported by the Carnegie Corporation of New York and by the Rockefeller Foundation. (Received for publication May 5, 1941.)

nutritional factors are involved in their etiology, it was hoped that the present study might contribute some basic information on the extent of the inorganic mineral metabolism of the teeth and their supporting tissues.

The attempts of Hevesy, Holst and Krogh (2) and Manly, Hodge and Van Voorhis (3) to study the radiophosphorus distribution of the component tooth tissues have yielded inconclusive evidence with respect to enamel. Recently, Hevesy and Armstrong (4) and Volker and Sognnaes (5) have published preliminary notes on the relative P^{32} distribution of the component tooth tissues, including the enamel, and the present report is a further report on the researches of the latter investigation.

PROCEDURE

The isotope P^{32} was prepared by the physics department of the University of Rochester in the university cyclotron. P^{32} thus prepared has a half life of 14.5 days. Nine adult cats, weighing 5 to 7 pounds, 5 adult dogs weighing 24–40 pounds, and 1 young adult monkey weighing approximately 8 pounds, were included in the experimental study. The experimental period was 1 to 9 days in the cat series, 4 hours in the dog series, and 6 days in the monkey experiment. The general procedure was to administer a known concentration of the radioactive phosphorus in a solution of Na_2HPO_4 containing 10 mg. or less of phosphorus.

The cats received a solution of the radiophosphorus by stomach tube which contained from 400,000 to 3,000,000 counts per minute and the monkey one containing 750,000 counts per minute in a similar manner. In the dogs a solution of 2,500,000 to 4,000,000 counts per minute of the isotope was administered directly into an isolated loop of the small intestine. At the indicated time the animals were sacrificed and the dentition, the supporting maxillary and mandibular bone, and in a few cases the femur, were taken for analyses. The samples were cleaned of adherent material, dried in an oven at 110°C. for 3 hours and pulverized in a mortar. The enamel and dentin were separated by the centrifugation-flotation method of Manly and Hodge (6). Since the distribution of radiophosphorus within the enamel is of special interest, this is studied in detail and is reported elsewhere.

Aliquots of the pulverized bone, whole teeth and purified enamel and dentin were dissolved in 6 N HCl and the radioactivity was determined on the Geiger-Müller scale-of-four counter and the accepted method used to calculate the radiophosphorus distribution in the tissues.

FINDINGS

The findings in the cat series are seen in Table I. No significant differences were observed that may be attributed to the varying experimental periods used in this series. With one exception the percentage of the dose found in the whole dentition varies from approxi-

TABLE I

Radiophosphorus distribution in the teeth and bones of the cat

CAT NO.	EXPER. PERIOD DAYS	PER CENT TOTAL DOSE IN WHOLE DENTITION	PER CENT TOTAL DOSE PER GRAM OF TISSUE				
			Enamel	Dentin		Mandible	Maxilla
				Crown	Root		
1	9	.0441	.0026	.0153	.0215	.0445	.0651
				Maxillae	Mandible		
2	8	.0208	—	.0082	.0084	.0140	.0232
				Crown	Root		
3	2	.0502	.0022	.0185	.0280	.0314	.0369
4	4	.0270	.0022	.0117	.0166	.0140	.0210
5	6	.0430	—	.0169	.0221	.0263	.0364
				Mixed			
6	2	.0514	—	.0323		.0486	.0749
7	1	.1681	.0035	.0789		.0860	.1433
8	3	.0190	.0012	.0086		.0130	.0162
9	6	.0395	.0016	.0179		.0245	.0425

mately 0.02 to 0.05 per cent. A comparison of the coronal dentin with the root dentin shows that the latter has 1.5 times the P^{32} content of the former. The radiophosphorus content of the maxillary bone is consistently higher than that of the mandibular bone, but the radioactivity of the dentin taken from upper and lower teeth is of the same order. Because of the low P^{32} concentrations of some of the experimental doses, and the small magnitude of the radiophosphorus turnover in the enamel, some of the enamel findings were inconclusive and have been disregarded. From the available data it appears that the P^{32} deposition of the enamel is approximately one-eighth that of the dentin.

Table II contains results observed in the dog experiments. Approximately 0.07 per cent to 0.12 per cent of the total dose could be found in the whole dentition. Here again the maxillary bone contains greater amounts of P^{32} than the mandibular bone and the root dentin more of the isotope than the coronal dentin. The values for the femur shaft are of the same magnitude as those of the maxillae, but show

TABLE II

Radiophosphorus distribution in the teeth and bones of the dog

DOG NO.	PER CENT TOTAL DOSE IN WHOLE DENTITION	PER CENT OF TOTAL DOSE PER GRAM OF TISSUE					
		Enamel	Dentin		Maxilla	Mandible	Femur shaft
			Crown	Root			
1	.072	.00015	.0015	.0025	.0048	.0034	—
2	.121	.0005	.0036	.0062	.0118	.0056	.0098
3	.091	.0002	.0031	—	.0071	.0038	.0051
4	.068	.0002	.0024	—	.0045	.0031	.0023
5	.092	.0004	.0042	.0054	.0064	.0043	.0033

TABLE III

Radiophosphorus distribution in the teeth and bones of the monkey

TISSUE	PER CENT TOTAL DOSE PER GRAM OF TISSUE
Dentin and cementum	0.0050
Enamel	0.0007
Unerupted teeth	0.0202
Erupted teeth	0.0052
Mandible	0.026
Maxillae	0.023
Femur epiphysis	0.037
Per cent total dose in whole dentition	0.0606

considerable variation from animal to animal. The radiophosphorus concentration per gram of tissue of the coronal dentin is approximately ten times that of the whole enamel.

The results with the monkey experiment may be seen in Table III. In this case 0.06 per cent of the total dose could be found in the whole dentition. The samples of the maxillary bone show only slightly higher values than those of the mandibular bone. A comparison of the radio-

phosphorus content of the erupted dentition with that of 4 unerupted partly calcified third molars shows the latter to have a 4 times greater P^{32} metabolism and one that is comparable to the surrounding bone. Samples of the femur epiphyses show the greatest concentration of the isotope found in this experiment. The enamel of the erupted teeth had approximately one-seventh the radiophosphorus deposition of the dentin from the same teeth.

DISCUSSION

The differences observed between the dog and cat series in the values for the percentage of total dose in the whole dentition, may possibly be accounted for by differences in the ratios of the dentition weight to body weight. This is approximately 3 times as great in the dog as in the cat and the same relationship holds for the percentage of total dose found in the whole dentition. It is also possible that the variation is related to the dissimilar experimental periods and the manner of the isotope administration.

It was observed that cats No. 7 and No. 8, which had the highest and lowest radiophosphorus deposition, were the youngest and oldest experimental animals used in this phase of the investigation. In cat No. 7 the root ends in some of the permanent teeth were incompletely calcified and large pulp chambers were still present. This condition indicates that a greater amount of the tooth substance has increased access to the circulating fluids containing the radioactive isotope. With cat No. 8 the reverse was true, as would be expected, since this animal had lost most of its anterior teeth because of advanced age, and since the pulp chambers had receded completely from the crowns of the remaining teeth.

The high concentration of radiophosphorus observed in the unerupted teeth than in the erupted teeth is probably an indication of the more adequate nutrition supply of the former. In the unerupted teeth the enamel was surrounded by the enamel epithelium and the dentin was in close proximity to the pulp chamber which at this stage in the tooth development occupies a large portion of the tooth crown.

The greater radiophosphorus deposition in the root than in the crown dentin may be the result of: 1. the inclusion in the former of the cementum. The cementum has a system of canals by which fluids may

pass from the periodontal membranes into the superficial and deep strata of secondary dentin which continues to be deposited throughout life. 2. It may be the result of an active exchange of the root dentin phosphorus with the P^{32} of the surrounding alveolar bone. This latter possibility is supported by the similarity observed between the radiophosphorus content of the root dentin and the maxillae.

The differences in P^{32} deposition in the various bone samples is probably an indication of the adequacy of their blood supply. The ratio of spongy to compact bone is considerably greater in the maxilla than in the mandible, and presumably spongy bone has a more adequate blood supply than compact bone.

SUMMARY

A study has been made of the radiophosphorus deposition in the bones and teeth of 9 cats, 5 dogs and 1 monkey. The cats, sacrificed from 1 to 9 days after administration of the isotope, had approximately 0.02 to 0.05 per cent of the total dose in the entire dentition. The corresponding values for a 4-hour experiment with dogs was 0.07 per cent to 0.12 per cent and for a 6-day experiment with a monkey 0.06 per cent. Per gram of tissue, enamel contained 10 per cent to 14 per cent of the radiophosphorus content of the dentin. In general, coronal dentin contained less marked phosphorus than root dentin and maxillary bone greater concentrations of radiophosphorus than mandibular bone.

The authors gratefully acknowledge the technical assistance of Dr. W. Bale and John Bonner of the Department of Radiology.

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RATE OF INCREASE OF DENTAL CARIES IN 707 OFFICERS AND MEN ABOARD AN AMERICAN MAN OF WAR[1]

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Much has been written concerning distribution and rate increase of dental caries under varying conditions (1, 2, 3, 4, 5, 6). However those observations were made on children that could be studied over a year period to determine rate increase of caries. The rate increase of dental caries in adults was calculated by comparing the variation of tooth destruction in different age groups rather than by direct analysis (2). The first *direct* analysis had been made in the Navy (8).

Dental examinations were made by clinical means only in all of the investigations cited except aboard the U.S.S. HOUSTON (8), where clinical and a combination of clinical and roentgenological means were employed. It has already been shown that "both clinical and roentgenological examinations failed in diagnosis of 24 per cent of cavities demonstrated in microscopic preparations" (7). It was interesting therefore to note what variation existed when clinical and roentgenological means were used for examination.

PURPOSE

Because of the possibility of error in using one method only for dental examination the purpose became clear immediately. It was decided to determine 1. what variations existed in caries detection when clinical and a combination of clinical and roentgenologic methods of examination were used, and 2. what influence such ways of examination would have on the rate increase of dental caries in a group of adults.

[1] (Received for publication June 9, 1941.)

[2] Lieutenant, Dental Corps, U. S. Navy.

PROCEDURE

A group of 707 officers and men aboard the U.S.S. HOUSTON were divided into a sub-group of 498 who were examined by clinical means only and a sub-group of 209 who were examined clinically and roentgenologically. Ages ranged from 17 to 51 years of age. By clinical

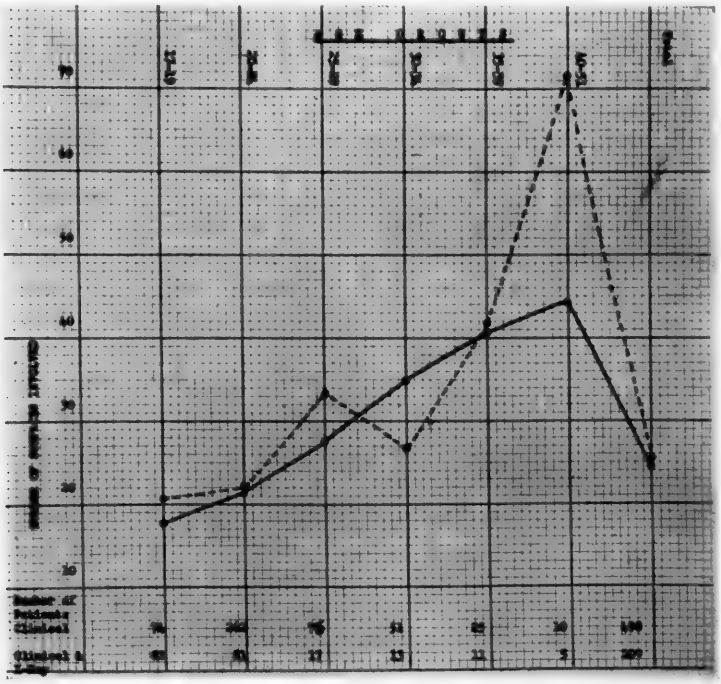

FIG. 1. Number of surfaces involved at first examination. ——— clinical examination; -- ---- clinical and x-ray examination.

examination is meant the use of a clear mirror, sharp explorer and transillumination. By a combination of clinical and roentgenologic examination is meant the use of the clinical means described and in addition the use of bite-wing roentgenograms.

Data of two examinations made on the same patients in each subgroup were used, that of an initial examination, and that of an exam-

ination 1 year later. Thus the same 707 officers and men were kept under dental observation for a full year.

Evaluation of tooth destruction was made by surface and included restorations as well as carious areas. By this means the total tooth destruction by number of surfaces was evaluated at the first examination, and repeated 1 year later. The caries increase was then evaluated by subtracting the number of surfaces involved at the initial examination from those a year later.

RESULTS

Tables I and II and *figs. 1* and *2* show that in all age groups except that of age 30 to 34, a greater number of tooth surfaces were found at-

TABLE I

First examination (caries, restorations, [extracted teeth, three surface evaluation]; by surfaces)

	AGE 17–19		AGE 20–24		AGE 25–29		AGE 30–34		AGE 35–39		AGE 40–51		TOTALS	
	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.
Clinical.....	74	17.8	262	21.3	76	27.4	51	34.8	25	40.5	10	44.1	498	24.5
Clinical and X-Ray.....	82	20.5	81	21.9	17	33.1	13	26.6	11	41.6	5	71.6	209	25.1

tacked by caries where a combination of clinical and roentgenographic means were used for examination. Totals show 0.6 surfaces per patient more tooth destruction in that group examined by clinical and roentgenographic means than in that examined by clinical means only at first examination.

After a 1 year period that group examined by clinical and roentgenographic means combined appeared to have less caries increase for each age group than the corresponding age group in that group examined clinically. Totals show $3\frac{1}{3}$ times less apparent caries increase (0.29 surfaces per patient) in that group examined by a combination of roentgenographic and clinical means than that group examined clinically only (0.85 surfaces per patient).

TABLE II

Caries increase in one year period by surface

	AGE 17–19		AGE 20–24		AGE 25–29		AGE 30–34		AGE 35–39		AGE 40–51		TOTALS	
	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.	No. pts.	No. surf.
Clinical.....	74	0.76	262	0.95	76	0.94	51	0.57	25	0.24	10	1.10	498	0.85
Clinical and X-Rays.....	82	0.56	81	0.56	17	0.00	13	0.00	11	0.00	5	1.00	209	0.29

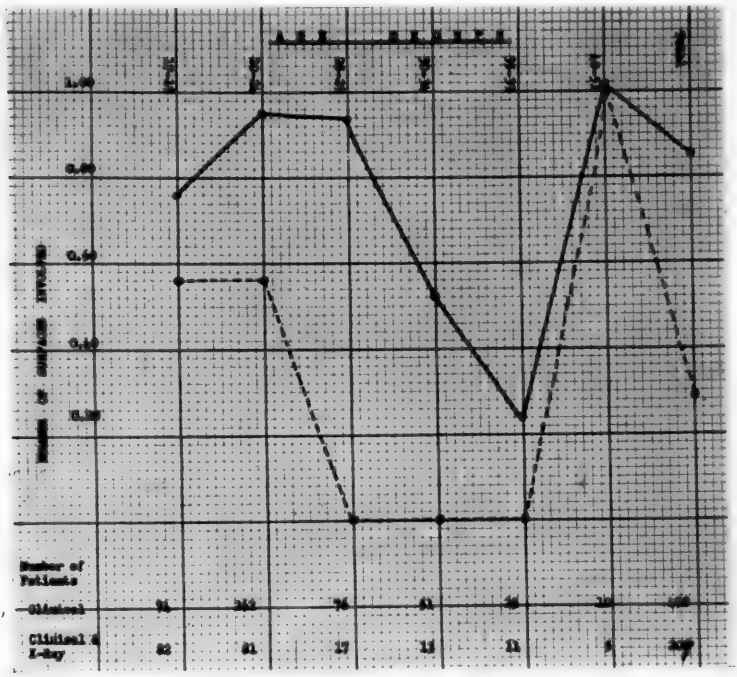

FIG. 2. Number of additional surfaces involved in one year (caries increase). ——— clinical examination; ------- clinical and x-ray examination.

CONCLUSION

Because of the small number of patients studied thus far no definite assertion can at this time be made on the influence method of examina-

tion has on caries prevalence at first examination and rate of caries increase. However this preliminary work seems to point to the suggestion that present rate increase of dental caries must be re-examined because more accurate initial examination tends to diminution of apparent rate of caries increase.

The opinions or assertions contained in this article are the private opinions or assertions of the writer, and are not to be construed as official or reflecting the views of the Navy Department or the Naval service at large.—Article 113, U. S. Navy Regulations.

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LOCAL MICRODETERMINATIONS OF GLUCOSE IN PERIODONTAL DISEASE[1]

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Local determination of blood constituents in gingival tissues is a new phase of periodontal investigation. The following report is based upon a study to establish the relative concentrations of gingival and systemic blood glucose and their modification by periodontal disease. This work is a continuation of two previous studies. Miller and Pelzer (1), showed that phosphatase from both finger and gingival blood is related to the speed of alveolar disintegration as determined from radiographs. They revealed that the more rapid the alveoloclasia, the higher the phosphatase concentration in the blood obtained from the gingiva in that area and the lower the concentration in the blood from the finger. Thus, gingival blood phosphatase was lowest in those cases with normal alveolar crests, or with arrested destruction, intermediate in the group with slowly active alveoloclasia, and highest where there was rapid destruction. Since the reverse relation existed for the finger blood, the greatest difference between finger and gingival phosphatase values lay in the rapid alveoloclasia group while the least difference existed in the arrested group; the slowly active series occupied an intermediate position. In another study, by the present authors in collaboration with Wolf (2), a number of young women who exhibited advanced alveoloclasia were subjected to complete physical, oral and laboratory examinations. The results of this study pointed to certain systemic characteristics common to almost the entire group. Among these characteristics were a somewhat elevated calcium level, a diminished phosphorus concentration, a lowered basal metabolic rate and specific dynamic action of proteins, a depressed phosphatase activity and a very much flattened glucose tolerance curve.

[1] (Received for publication May 3, 1941.)

[2] Research Associate through grant from Bristol-Myers Co.

It will be noted that the diminished phosphatase obtained in the latter study agrees substantially with the result obtained in the first study in which systemic, i.e., finger phosphatase was found to be lowered in cases of slowly and rapidly active alveoloclasia. Thus, it seemed probable that deviations in other blood constituents would be found in the gingival tissues surrounding bone undergoing destruction. One of the known causes for a flat sugar tolerance curve is an increased ability to store the glucose. The question arose whether this substance was not being excessively stored in the periodontal tissues undergoing destruction.

METHOD OF STUDY

Ninety-five cases, selected at random, were studied. A gingival area, usually in the upper right, either between the first premolar and the canine, or between the 2 premolars was selected. The clinical appearance of the gingiva was noted and was characterized according to form (interproximal destruction, edematous, hypertrophied, normal, etc.) and color (red, purple, normal, etc.). The relative amount of bleeding produced by the incision was noted numerically: 1, scant; 2, moderate; 3, profuse; and 4, very profuse. There was definite relation between the color and form of the gingivae and the amount of bleeding produced by the incision, eg., pale gingivae bled the least while purplish and edematous tissues bled most profusely. Approximately one-third of the cases selected for this work had to be eliminated because the incision failed to elicit sufficient bleeding. The remainder, 95 cases, represent those in whom an incision produced at least 75 cubic millimeters of blood (50 are required for the quantitative analysis).

The gingival blood sample was obtained by first thoroughly drying the area including the mucosa and adjacent teeth. This was done to prevent contamination and dilution of the blood sample by any of the oral fluids. In addition, drying the area facilitated obtaining the sample since it insured a spherical accumulation of the blood because of surface tension. The papilla between the 2 teeth was incised to the height of the alveolar crest with a sharp scalpel without anesthesia. No attempt was made to remove the papilla. Fifty cubic millimeters of the resulting blood were drawn into an accurately calibrated pipette preferably by capillarity. The sample was then vigorously discharged into a centrifuge tube containing 2,500 cubic millimeters of a freshly

prepared dilute precipitating mixture.[3] The pipette was rinsed with this mixture at least 3 times. The tube was then stoppered and shaken vigorously for 1 minute.

The same procedure was followed for the finger. The palmar surface of the terminal phalanx of the middle finger was washed with alcohol, dried, and punctured with a sharp spring lancet to a depth of 3 millimeters.

Both tubes, containing finger and gingival blood samples, were then immediately centrifuged and quantitative glucose determinations were run on 2 milliliters of the resulting clear supernatant fluid. Benedict's microglucose technique was employed.

RESULTS OF STUDY

The mean finger glucose $\left(\frac{\Sigma X}{N}\right)$ value of the total number of cases was found to be identical with the mean gingival glucose $\left(\frac{\Sigma Y}{N}\right)$ value. In both cases it was 83.9 mgms. In addition to the recording of the aforementioned information, the percentage difference between the gingival and the finger values (Z) was computed by the formula: $Z = \frac{100\,Y}{X} - 100$, in each case a plus figure indicated that the gingival (Y) value was greater than the finger (X) value. A frequency distribution curve (*fig. 1*) of the percentage differences for the entire group of 95 cases very closely followed a normal, or bell curve which would indicate that the variations in these figures occurred by chance. The mean percentage difference $\left(\frac{\Sigma Z}{N}\right)$ was computed to be +.5 per cent while the limits of error of the technique employed were from 1.5 to 2 per cent.

For purposes of analysis of the data obtained, the 95 cases were classified first according to bone type, or speed of alveolar destruction, then according to the relative amount of bone disintegration, and finally, according to the amount of bleeding elicited by the incision.

There were 31 cases of arrested destruction; the mean difference

[3] Prepared by the addition of 150 milliliters of distilled water to 5 ml. tungstomolybdic acid in a 250 ml. volumetric flask. To these is added 5 ml. of .62N sulfuric acid and dilute to the mark with distilled water. The mixture is stable for 3–5 days.

$\left(\frac{\Sigma Y}{N} - \frac{\Sigma X}{N}\right)$ between the gingival and the finger values was .8 mgms. while the mean percentage difference $\left(\frac{\Sigma Z}{N}\right)$ between the two was −2.2 per cent. Fifty eight cases of slowly active disintegration had a mean difference between X and Y of −.3 mgms. while the mean percentage difference was −.2 per cent. The group showing rapid destruction comprised 6 cases and had a mean difference between the 2 samples $\left(\frac{\Sigma Y}{N} - \frac{\Sigma X}{N}\right)$ of −.2 mgms. while the mean percentage difference $\left(\frac{\Sigma Z}{N}\right)$

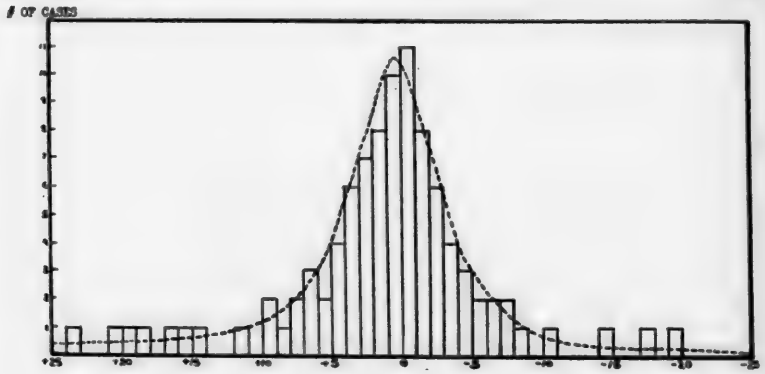

FIG. 1. Frequency distribution curve of percentage differences between finger and gingival blood glucose values of all 95 cases. Note that curve obtained very closely resembles a normal, or bell curve.

was −.3 per cent. From Table I it will be seen that the computed standard deviation (σ) values are so large as to make comparative figures of the 3 subgroups not significantly different.

The series was then classified according to the amount of bone destruction seen in the radiograms. The amount of destruction was recorded by a series of numbers from 0 to 5; 0, where no disintegration was seen in the particular area; 5, where the destruction had gone as far as the apices of the teeth. A unit of 3 was ascribed to the area when half of the bone had disappeared. Units of 1, 2 and 4 were estimated as fractions of 3 and 5. Because there were so few cases in the extreme destruction range, those showing either 4 or 5 destruction were classified together. It will be noted from Table I that when the cases

are classified in this way, there is no difference in comparable values; that all the finger (X) glucose values are probably identical since the standard deviation, σ_x, is so large. The same is true for gingival (Y) glucose values. Because the mean percentage differences $\left(\frac{\Sigma Z}{N}\right)$ are

TABLE I

The 95 cases were classified according to speed of alveolar disintegration (arrested, slow and rapid), according to the amount of alveoloclasia (0 bone destruction, 1, 2, 3 and 4 and 5 bone destruction) and according to the amount of bleeding produced by the incision (1 flow, 2, 3 and 4 flow).*

	s	ϵX	$\frac{\epsilon X}{N}$	σx	ϵY	$\frac{\epsilon Y}{N}$	σy	$\frac{\epsilon Y}{N}-\frac{\epsilon X}{N}$	ϵZ	$\frac{\epsilon Z}{N}$	σz
Arrested.....	31	2671.3	86.2	±26.8	2696.3	87.0	±22.7	+.8	+68.9	+2.2	±7.2
Slow.....	58	4783.5	82.5	±8.2	4764.9	82.2	±11.6	−.3	−12.4	−.2	±7.5
Rapid.....	6	514.7	85.8	±12.9	513.3	85.6	±12.5	−.2	−1.7	−.3	±1.4
0 B.D.....	35	2892.0	82.7	±25.1	2907.2	83.0	±22.8	+.3	+53.0	+1.5	±7.4
1 B.D.....	31	2536.7	81.8	±11.4	2558.7	82.5	±10.7	+.7	+45.5	+1.5	±8.0
2 B.D.....	18	1577.2	87.4	±15.5	1541.8	85.6	±15.3	−1.8	−48.7	−2.7	±7.0
3 B.D.....	9	811.6	90.2	±12.3	814.5	90.4	±8.5	+.2	−11.9	−1.3	±4.1
4–5 B.D.....	2	152.0	76.0	±17.4	152.3	75.7	±15.3	−.3	+.2	+.1	±2.4
1 Flow.....	18	1416.0	78.7	±11.8	1403.3	77.8	±4.3	−.9	−6.2	−.3	±9.9
2 Flow.....	32	2825.4	88.3	±25.9	2818.9	88.1	±21.9	−.2	+19.3	+.6	±5.2
3 Flow.....	37	3015.9	81.5	±13.8	3058.5	82.6	±10.9	+1.1	+46.1	+1.2	±7.7
4 Flow.....	8	712.2	89.1	±12.8	693.8	86.5	±12.1	−2.6	−22.0	−2.8	±4.9
Totals.....	95	7969.5	83.9	±18.8	7974.5	83.5	±16.7	0	+54.9	+.5	±7.3

* X denotes finger glucose values; ϵX, the sum of the finger blood glucose values in a particular subgroup; x, the standard deviation of the mean finger glucose value $\left(\frac{\epsilon X}{N}\right)$. Y denotes gingival blood glucose values. $\frac{\epsilon Y}{N}-\frac{\epsilon X}{N}$ is the gingival glucose value minus the mean finger glucose value. Z denotes the percentage difference between the finger and the gingival values. A positive figure indicates that the gingival value is greater. Note that the mean values for each subgroup are very close and that the standard deviations (σ) are so large as to make them not significantly different.

very close to the limits of experimental error and because the standard deviations of the means (σ) are so large, all of the values, finger and gingival, are almost certainly not significantly different.

It would seem reasonable to expect that with changes in color of the gingiva, i.e., degree of inflammation and consequent stasis, alterations in glucose concentration would be observed. Since color

and form were directly correlated with the amount of bleeding produced by the incision, it was preferred to use the latter since it was much more definite and objective. The results obtained when the group was classified in this way are no more significant than with the other 2 classifications (Table I).

The lack of any definite results may be due to a number of factors chief of which is the impossibility of obtaining a sample of which a significant portion is related to the disease process. The proportion of the related blood to the total blood in the sample is an unknown and probably an inconstant factor.

SUMMARY

1. Ninety-five cases, selected at random, were studied to determine whether alterations occurred in either finger or gingival blood glucose concentrations in periodontal disease.

2. The group was classified first according to bone type or speed of alveolar destruction, then, according to the relative amount of bone disintegration, and finally, according to the amount of bleeding produced by the incision.

3. All of the finger and gingival glucose values had percentage differences between them too close to the limits of error of the technique to be significant.

4. The study reveals that there probably is no alteration in glucose concentration if the method utilized is a tenable one.

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THE EFFECT OF MATERNAL DIETARY DEFICIENCY OF VITAMIN A ON DENTAL TISSUES IN RATS*

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Several workers, including in particular May Mellanby (1920, 1928, 1929) and Toverud and Toverud (1931) have clearly shown that deficiency of fat-soluble vitamins and mineral salts in the maternal diet of animals has an adverse effect on the teeth of their young. The present work deals solely with a deficiency of vitamin A, the rest of the diet being made as complete as far as is known, and extends observations already published (H. Mellanby, 1939).

The dentition of a rat consists of one pair of incisors and 3 pairs of molars in each jaw (*fig. 1*). Only one set of teeth is developed and the incisors grow persistently while the molars are rooted. Eruption of the teeth begins with the incisors at the age of 10 to 11 days, these are followed by the first and second pairs of molars about the 17th to 18th day, while the third molars do not erupt until the rats are 5 weeks old. When a rat has reached the age of 6 to 7 weeks its molar teeth, including their roots, may be considered to be fully developed.

The age at which a rat's molar teeth have completed development is important when considering the effect of diet on these teeth. Young rats are normally weaned at the age of 3 weeks, by which time the crowns of the first and second molars are almost fully formed. An experimental diet begun at 3 weeks could not be expected to produce much effect on the molar teeth, especially when the animal's own reserves of vitamin are often sufficient for normal development to proceed for a further 2 to 3 weeks. Any abnormal development under these conditions would be most likely to occur in the roots of the molars and perhaps to some extent in the 3rd molar crowns. The whole question is complicated by the quality of the stock diet on which the mothers and young were fed until the latter were weaned. Abnormalities in molar root structure of rats have been observed by Becks and Ryder (1931) who fed young rats on a post weaning rachitogenetic diet (McCollum's diet no. 3143).

The abnormal structure seen in rat's incisor teeth, when growing and when adult rats are fed on a vitamin A deficient diet, have been the subject of study by various workers including Wolbach and Howe (1933), Fridericia and Gudjonsson (1936), and Pohto (1938). The present work confirms and extends their findings, but the typical

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changes were observed to take place much earlier in the rat's life and in some cases had reached an advanced state before the young were weaned or the teeth erupted. The molar teeth were also much affected by vitamin A deficiency in the maternal diet, though even in these experiments the effect was less than that observed on the incisors. An interesting feature of all the experiments to be described below is that the abnormalities present in the teeth of the baby rats (incisors) were always much more marked than those present in their mothers' teeth. Thus it would appear that with this particular dietary de-

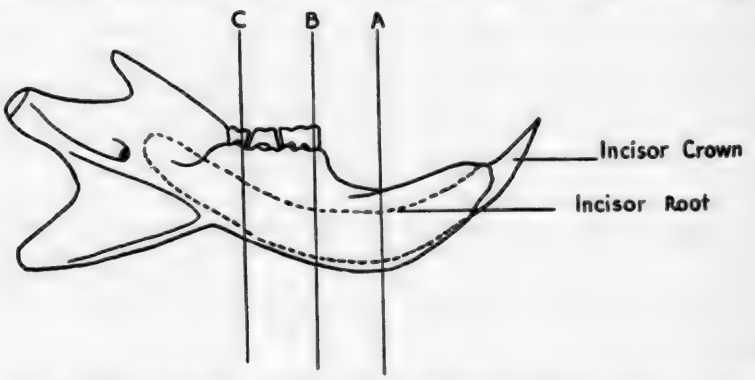

FIG. 1. Diagram of left mandible of albino rat showing position of teeth. Vertical lines indicate approximate planes of sections photographed in this paper.

ficiency the offspring suffer more than the mother; the reverse of this state of affairs is thought to be true for mineral salt deficiency in some animals (Theiler, 1934).

METHODS

For each series of experiments a number of female rats, all litter mates, were placed on the experimental diet when they were aged 3 to 4 weeks. The diet was deficient in vitamin A though not absolutely devoid of it, so that the rats survived for fairly long periods, up to 40 weeks on diet in some cases, though estimation of the liver stores of vitamin A showed that these were exhausted after 12 to 15 weeks on diet, whereas the control animals had between 100 and 300 blue units per gram of liver. In each litter of experimental females one or two animals (depending on the total number of females) were given in addition to the diet a concentrate of vitamin A[1] at the rate of 100 Blue units per day and acted as controls.

[1] This was given in the form of Avoleum kindly supplied by Messrs. British Drug Houses Ltd.

Apart from the vitamin A deficiency the experimental diet appears to be complete. Five generations of control rats have now been reared on the diet plus a Vitamin A concentrate, and all these rats have been healthy, though their weight is somewhat below that of animals reared on a good stock diet which includes fresh milk.

The following are the details of the experimental diet. Each rat received daily 14 to 18 gms. of bread, 2 gm. of skim milk powder, 1 gm. of fresh baker's yeast, 0.2 gm. of sodium chloride, 0.3 cc. of lemon juice, and 0.5 cc. of 5 per cent solution of a Vitamin D concentrate (Radiostol) in olive oil, and in most experiments a daily dose of 0.015 cc. of wheat germ oil was given to insure an adequate supply of vitamin E for breeding. In addition 2 gm. of lean meat and 2 gms. of previously heated Alfalfa were given to each rat twice a week, and 2 gms. of marmite once a week. The controls received their supply of vitamin A in the form of concentrated mammalian liver oil (Avoleum). In the later experiments the use of wheat germ oil was discontinued and a synthetic form of vitamin E, tocopherol acetate was substituted.

After a definite length of time on diet, the litters of females were put to breed with stock males, care being taken to see that the vitamin A deficient females did not become contaminated with the feces of stock males. Each group of females was dieted for periods of between 12 and 34 weeks before breeding was attempted so that the various groups of females differed in their "deficiency" of vitamin A before the breeding was begun.

As soon as any female became pregnant she was placed in a separate cage standing on blotting paper, and she was weighed frequently to insure that any premature births did not pass unrecorded. This was particularly necessary in the case of rats which had been vitamin A deficient for a long time, since they tended to produce dead litters or abortions. When the young were born they were killed at various ages depending on the state of health, young from a control litter mate being killed at the same age for comparison. The young were weaned in 21 days, and then the mothers, both Vitamin A deficient and controls, were killed to provide material for a histological study of incisor teeth and gingival tissue to serve as a guide to the degree of their vitamin A deficiency. The livers from all the mother rats, many of the young rats, and also a few bodies from the young were estimated for their vitamin A content in Carr-Price blue units using a Lovibond Tintameter.

The jaws of both the mother rats and their young were fixed in 10 per cent formol saline either by perfusion through the heart, or by the dissected jaws being placed in the solution. The right half of the lower jaw was sectioned serially after being first decalcified in 2 per cent or 5 per cent nitric acid and then embedded in wax. In the young rats every third section was mounted, but in the mothers only every twentieth section was taken except in the molar region where every third was taken. The sections were cut 4 by 7 micra thick and were stained with Weigert's hematoxylin and counter stained with eosin or Bibrich scarlet.

EXPERIMENTS

Series 1. Females littering when 15 to 16 weeks old, after 12 to 13 weeks on vitamin A deficient diet

Seven female rats from one litter (Nos. 191–197) were placed on diet when they were 3 weeks old, 2 of them (191 and 192) being given

vitamin A and acted as controls. After 12 to 13 weeks on diet, litters were obtained from them. One control litter and one vitamin A deficient litter were eaten by the mothers and one vitamin A deficient litter was stillborn. The remaining 4 litters survived and provided a total of 32 young. None of the young from the vitamin A deficient mothers showed any abnormality in the structure of their teeth up to the age of 5 weeks. Several of these young born of deficient mothers were reared for periods of 2 weeks with a control rat as a foster mother; this resulted in their livers containing detectable amounts of stored vitamin A as compared with none in 8 out of 9 of their A deficient litter mates.

Two female young from one of the A deficient mothers were allowed to remain alive until they became moribund at the age of 30 weeks. An attempt to get them to breed resulted in both individuals containing macerated full term fetuses when they were killed. At the time of death these individuals had developed abnormal incisor teeth, and the gingival epithelium of the molars showed changes similar to those described by King (1940).

The quantity of stored vitamin A present in the livers of the vitamin A deficient mothers at the end of lactation (when they were killed) varied between a trace and 3 blue units per gram. These rats were then 18 to 19 weeks old and had been 15 to 16 weeks on diet. In the liver of only one out of 9 young reared with their own A deficient mothers was there found a trace of vitamin A, all the rest examined had none. Young (5 individuals) reared with a control rat as a foster mother were found to have an average of 3 blue units per gram of liver when killed at 4 to 5 weeks old. The two control mother rats had 90 and 160 blue units per gram of liver.

Conclusions from Series 1. It is possible to obtain what appear to be normal young from females whose store of vitamin A is on the point of exhaustion. These young also seem capable of normal development for some weeks at least, which confirms the work on Baumann, Riising and Steenbock (1934) who found that the period of growth in rats was longer than could be predicted on the basis of liver stores of vitamin A. When two such young were kept on the diet indefinitely, death occurred at the age of 30 weeks.

Series 2. Females littering when 19 and 22 weeks old after 15 and 19 weeks on vitamin A deficient diet

Three female rats (Nos. 211, 212 and 213) were placed on diet at the age of 4 weeks, one of them being given Vitamin A to act as a control. After 15 weeks on diet, No. 212 (vitamin A deficient) littered, while the other 2 rats (the control and the second vitamin A deficient) littered on the same day after 18 weeks on diet. The latter two families were the same size and with the same proportion of the two sexes so they were carefully compared as regards their weight curves, clinical condition and histology when killed.

Sections were made from 3 young of rat 212 which littered after 15 15 weeks on diet. The young were 3, 8 and 19 weeks old. At the age of 3 weeks the incisor teeth showed slight abnormalities near the root, but the anterior part of the incisor, as well as the first two molars appeared normal. In the 8 and 19 week old young the incisor showed much greater abnormalities. An attempt to get the one killed at 19 weeks old to breed when it was 16 weeks old resulted in the fetuses dying in utero.

The second 2 families born after their mothers had been 19 weeks on diet will be considered in more detail. Both the control family and the vitamin A deficient consisted of 10 babies and the families were reduced to 6 each at the age of 7 days so that there would not be too many for the mother rats to look after. More young were killed aged 3, 16, 17 and 18 weeks.

In the molar teeth the vitamin A deficient young showed no abnormalities at the age of 7 days, but in all young aged 3 weeks and over the roots of the molars were abnormal; the root dentin had an irregular outline and the actual root apex tended to become bulbous instead of having the normal almost square outline (see *fig. 2* for shape of molar root.) Those which survive for 16, 17 and 18 weeks were of course the most affected, and the roots of the third molars, being the last molars to develop, were the most abnormal. The gingival and subgingival epithelium of these molars was also affected by the vitamin deficiency. It became keratinised and thickened, the subgingival part tending to grow downwards alongside the cement; the sulcus contained some calculus-like debris. The results are similar to those observed by King

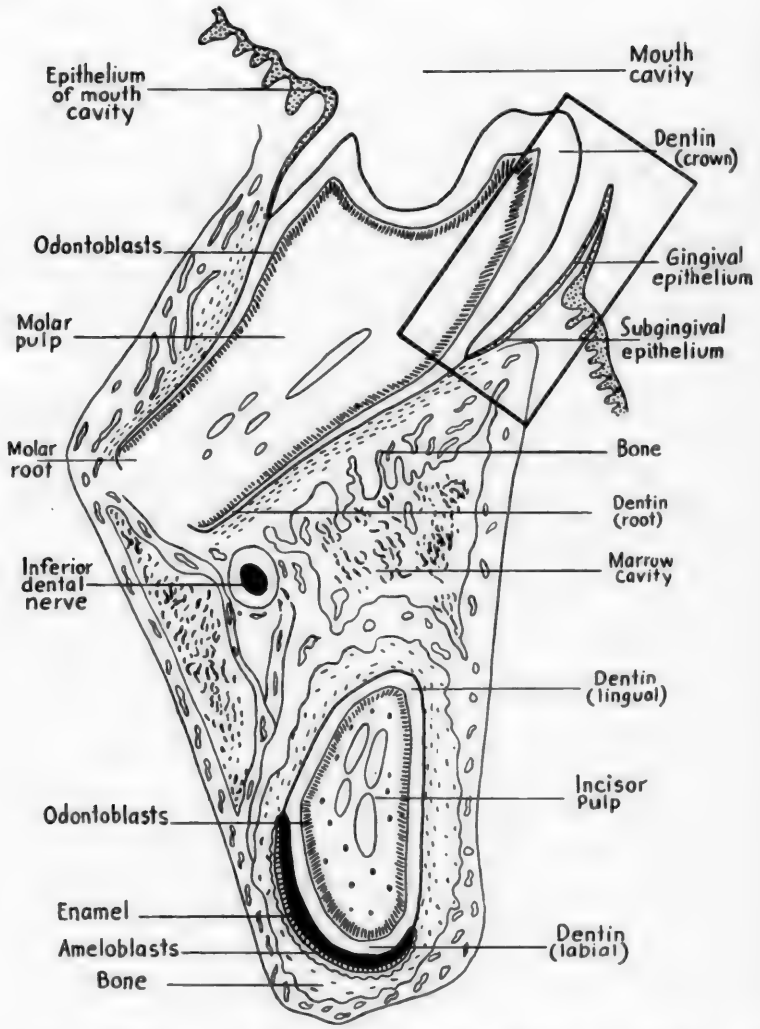

FIG. 2. Diagram of T.S. through lower incisor and 1st molar of a young rat. Area inside rectangle indicates region photographed to show molar gingival tissues. (N.B. The enamel of the molar has dissolved on decalcifying.)

The following abbreviations are used in subsequent illustrations: Am.—ameloblasts, B.M.—bone of mandible, D.—dentin, E.—enamel, K—keratinised epithelium, M.C.—mouth cavity, M.—molar root, Od.—odontoblasts, R.D.—root dentin, S.E.—subgingival epithelium.

(1940) in his study of abnormal gingival and subgingival tissues in rats on vitamin A deficient diets.

The incisor teeth of the vitamin A deficient young from rat 213 showed more spectacular abnormalities. Transverse sections of the

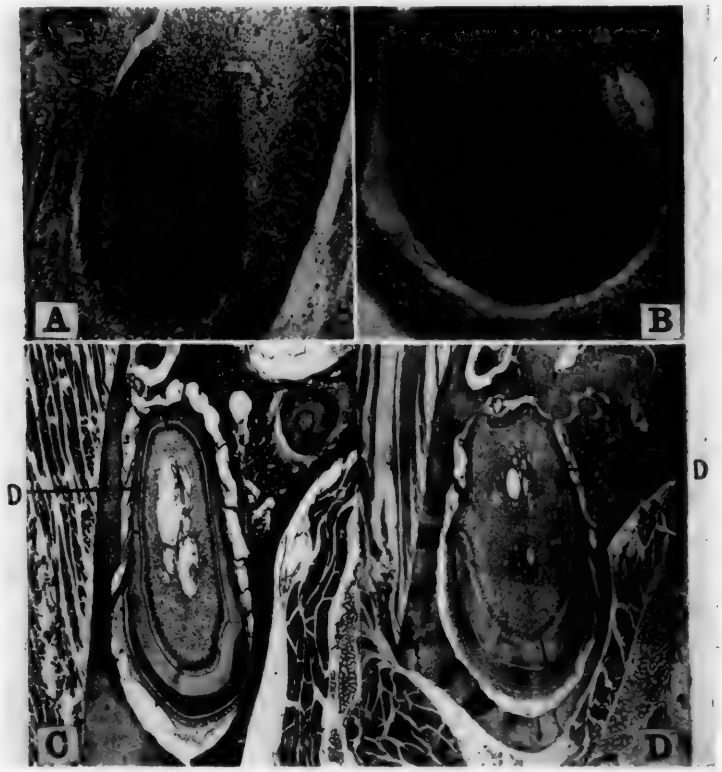

FIG. 3, A and B. T.S. lower incisor root in 7 day old rats. A. Root of control young of rat 211. B. Root of vitamin A deficient young from rat 213. × 90.

C and D. T.S. Lower incisor root on level of third molar in 17 week old rats. C. Young of control rat 211. D. Young of vitamin A deficient rat 213. × 40.

incisor roots of young aged 7 days showed that already there was considerable deviation from the normal. (Compare *fig. 3A*, normal, with *fig. 3B*, vitamin deficient.) It will be seen that the breadth of the root is increased and the shape of the outline differs from the normal. By the age of 3 weeks the difference was more pronounced,

while at 16, 17 and 18 weeks a cross section of the incisor in the lower third molar region (*fig. 3C, D*) showed pronounced abnormality.

The characteristic changes in the appearance of rat incisors after these animals have been fed on vitamin A deficient diets for varying periods are described by Wolbach and Howe (1933), Pohto (1938), and King (1939). First the shape of the tooth is abnormal; atrophy and metaplasia of the enamel organ occurs, and finally there is very irregular production of dentin which may be absent altogether in some places. All these abnormalities occurred among the young rats in this and the following experiments and the severity of the abnormalities depended on the length of the dieting period of the mother prior to the production of her family.

The abnormal shape of the rat's incisor in vitamin A deficiency would appear to be due to the failure of the enamel organ in the first place to outline the normal shape of the tooth (*fig. 3B*). In severe cases the enamel organ may be absent from areas on the lingual side of the tooth which leads to an absence of dentin at such places since the formation of dentin is controlled by the enamel organ (Glasstone 1936). In milder cases of deficiency the dentin is irregularly laid down and an uneven odontogenetic zone is produced. The lingual side of the tooth tends to become buckled.

Schour, Smith and Hoffman (1938) have shown that the change in shape which rat incisors undergo in Vitamin A deficiency is due to an unequal deposition of dentin on the labial and lingual sides of the tooth. Normally the dentin is of similar thickness on both aspects of the tooth, but on Vitamin A deficient diets the apposition of dentin is increased on the labial (enamel) side while on the lingual side it is decreased.

Another point to be noted about the tissues of the vitamin A deficient young in this and the succeeding experiments is that the jaw bone encroaches more closely on the incisor tooth than it does in the normal rat, and the area of bone between incisor and molar roots is much reduced (*fig. 6C, D*). On the whole the jaw bones of young rats, vitamin A deficient from birth, show increased density and fewer marrow spaces than the normal.

When the vitamin A reserves of the animals in this series were estimated the results were interesting. Female 212 killed after 18 weeks on diet had only a very faint trace of vitamin A in the liver.

Female 213, killed after 22 weeks on diet, had none. The control female had 37 B.U. per gram. Young from the control rat had the following amounts of stored vitamin A; age 1 week (average of 4 young) 21.5 B.U. per gr. of liver, age 3 weeks liver had 21 B.U. per gr. while the body contained an additional 1.3 units per gr. None of the young from the Vitamin A deficient females had any stored vitamin A.

The incisors and gingival tissues of the 3 mother rats were compared histologically. Those of the control rat, 211, were normal in every way, while those of the deficient mothers showed some defects in the incisor roots and molar gum tissue, but these were relatively slight.

Conclusions to be drawn from Series 2. When female rats aged 4 weeks at the beginning of the experiment have been on this particular vitamin A deficient diet for 15 weeks before their litter arrives, then the teeth of their young begins to be adversely affected by the vitamin deficiency before weaning. A litter produced later than this, when the female has been 19 weeks on diet, had their teeth markedly affected before weaning. Growth of the young however, though not so good as the controls, was not very much reduced, and death of the young from vitamin A deficiency was delayed until the young were between 16 and 18 weeks old. The vitamin A deficient mothers both showed slight abnormalities in their incisor roots and gingival tissues.

Series 3. Females littering when 28 weeks old after 24 weeks on diet

Three female rats were put on diet at the age of 4 weeks (Nos. 231, 232, 233). No. 231 was given mammalian liver oil as a control. All 3 rats littered after 24 weeks on diet. The litters were successfully reared, some young were killed aged 21 days, and the remainder when the A deficient young began to loose weight rapidly, between 6 and 10 weeks after birth.

At 7 and 21 days the vitamin A deficient young showed considerable abnormalities in their teeth. Transverse sections of 21 day rats taken on a level of the first lower molar showed the incisor, and to a lesser extent, the molar to be affected (*fig. 4A and B*). The incisor tooth was more abnormal than in the case of the 3 week young of rat 213 in *Series 2* which had been born after its mother had been 19 weeks on diet. From the 2 vitamin A deficient litters, 11 young were

examined histologically, and in all cases the teeth were very abnormal. The longest period of survival was 9 weeks in one A deficient family and 10.5 weeks in the other. When these were compared with control young of the same age the abnormalities in their teeth were very pronounced. Considering first a comparison of their molar teeth, *fig. 6A* and *B*, shows a transverse section of the second molar of a 9 week control compared with that from a 9 week old vitamin A deficient young. In the latter the crown of the molar is reasonably normal, but the gingival tissue and the roots are not. The gingival and subgingival epithelium show the typical vitamin A deficiency changes, though these are not particularly far advanced; they were much more abnormal in two of the rats which died younger at 7.5 and 7 weeks

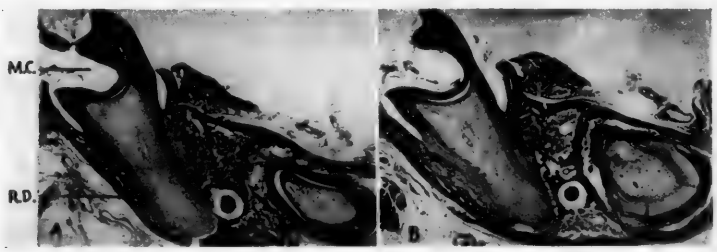

FIG. 4. T.S. Lower incisor and first molar in 3 week old rats. × 90. A. Young of control rat 231. B. Young of vitamin A deficient rat 233.

(*fig. 5B and C*). The gingival and subgingival epithelium is thickened and keratinised when compared with the control. Higher magnification photomicrographs of the gingival region in 2 young from the same and a sister vitamin A deficient mother (*fig. 5B and C*) show the subgingival epithelium growing down beside the cementum, and the general condition of the gum tissues was worse in these young. The molar roots in the case of one 9 weeks old A deficient young show several abnormal features, the outline of the roots is uneven, due to irregular deposition of dentin, and the root apices appear to be malformed (*fig 6B*).

A comparison of the incisor from 10.5 week old vitamin A deficient young with that of a control young of the same age shows how very abnormal the incisor of the former had become (*fig. 6C and D*). This section is taken on a level with the third molar and it will be seen that

the relative position of the third molar to the incisor is far from normal, this would appear to be caused partly at least, by the different rates of growth of the surrounding bone in the vitamin A deficient and the

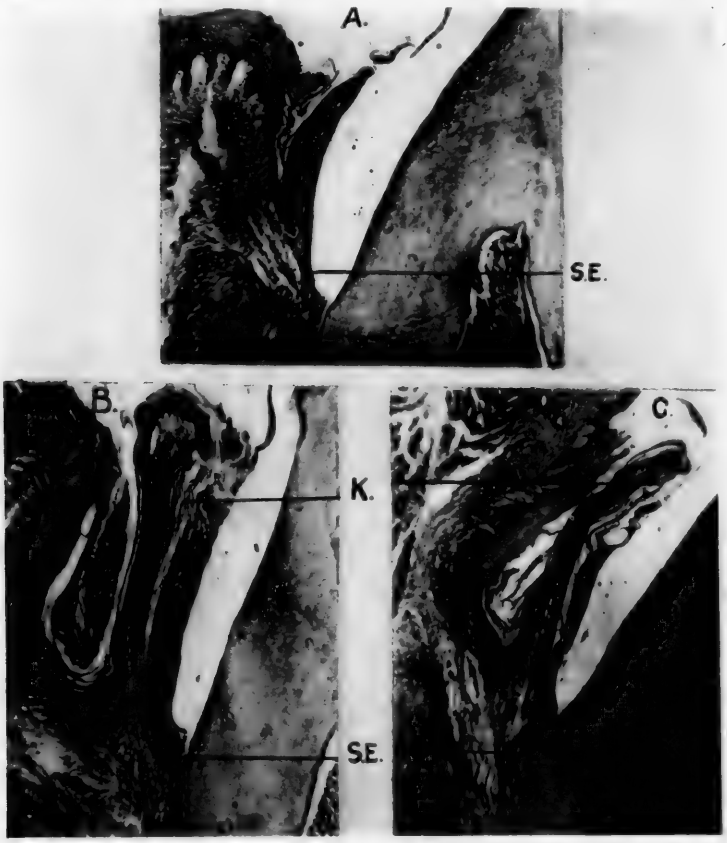

FIG. 5. T.S. of gingiva with subgingival epithelium from first lower molar of 7 week old rats. × 90. A. Young of control rat 231. B. Young of vitamin A deficient rat 233. C. Young of vitamin A deficient rat 232.

normal young. The control animal has large marrow spaces in the bone below the third molar and the bony socket surrounding the incisor does not impinge closely upon the tooth. In the vitamin A deficient young on the other hand, the bone below the third molar is

dense and contains only small marrow spaces, while in places bone impinges closely upon the incisor teeth. The incisor itself (*fig. 6D*) is broader than normal with little or no dentin on the lingual side, in

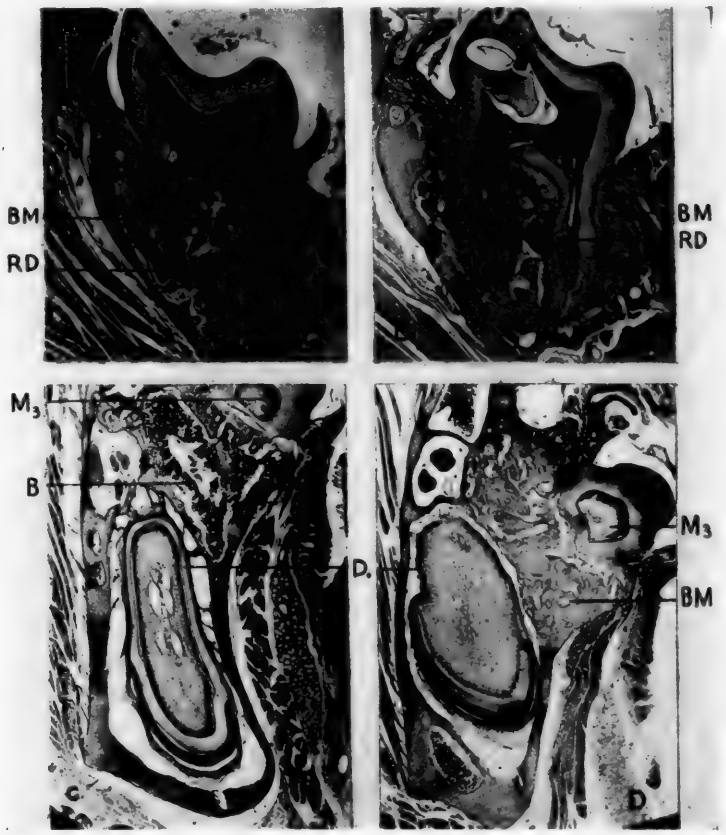

FIG. 6, A and B. T.S. of second lower molar in 9 week old rats. × 40. A. Young of control rat 231. B. Young of vitamin A deficient rat 232.

C and D. T.S. of lower incisors in 10.5 week old rats. × 40. C. Young of control rat 231. D. Young of vitamin A deficient rat 233.

fact the enamel organ has not enclosed all the lingual side of the tooth and where there is no enamel organ, there is, of course, no dentine. Hardly any of the odontoblasts have differentiated on this side, and the lingual part of the enamel organ has a wavy outline.

A comparison was made of the control and the A deficient mother rats when they were killed after 27 weeks on diet. In both deficient mothers the gingival and subgingival epithelium was hyper-keratinised and the subgingival epithelium had grown down alongside the cementum for some distance. The growth of the incisors was normal in all the length in front of the third molars, but some little way behind this the enamel organ on the labial side of one mother became frilled in appearance and was definitely not normal. However the condition of the mother rat's incisor teeth was far less abnormal than that of her young killed at the same time (i.e. when the young were 3 weeks old). Neither A deficient mother had any vitamin A in her liver at death. The control had 104 Blue units per gram of liver.

Conclusions from Series 3. The longer dieting period of 25 weeks (cf. *Series 2*) preceding the birth of the litters from 232 and 233, resulted in the teeth of their young being more profoundly affected than those of *Series 2* (19 weeks on diet). The shorter time of survival of the young as compared with those in *Series 2*, must be attributed to the same cause.

The gum tissues of both A deficient mothers and the incisors of one, were just beginning to show abnormalities at the time of killing (after 27 weeks on diet). Those young killed on the same day as the mothers were much more abnormal.

Series 4. Females littering when 28 and 33 weeks old after 25 and 30 weeks on diet

Six females (Nos. 241–246) were placed on diet at the age of 24 days. Nos. 241 and 242 were control animals, while 243–246 were vitamin A deficient. After 25 weeks on diet Nos. 241–244 produced litters. The two litters from 243 and 244 were rather weak and none survived longer than 17 days; before that age some died and others had to be killed as they were almost moribund. Material was obtained from 8 individuals, 4 of which were fixed by perfusion through the heart. Control young were killed at the same age. When material from these families was examined histologically it was found that the deficient young showed gross abnormalities of the tooth structure similar to those already described (H. Mellanby 1939). In *fig. 7C and D*, sections through the first lower molar and incisor in two rats at 17 days are shown for comparison. In the control the first molar is

just erupting, its root development is well advanced and the enamel has dissolved out on decalcification as it was almost fully formed. The first molar of the A deficient young has hardly any root and is very much delayed in its development; the enamel, being only partly calcified, has not dissolved out with the action of the acid, and has stained deeply. The lower incisors in the 2 cases are also very different, that of the control being normal for this age of rat, but that of the A deficient young very abnormal; as is seen by comparing the outline of the two teeth, the relative amounts of dentin deposited, the thickness of enamel, etc. In *fig. 7A and B*, transverse sections of the lower jaws of two 15 day old rats are shown. The section passes through the same developmental stage of the root end of the incisor, but in the control animal the incisor at this stage has grown back below the level of the third molar. In the A deficient young the incisor only extends back to the second molar region as its development has been retarded. Apart from this difference the shape of the root in the 2 is dissimilar showing that the enamel organ of the A deficient young fails to define the correct shape as it extends backwards to outline the root. All the young from the litters of rats 243 and 244 showed the abnormalities in their teeth which are depicted in *figs. 7B and D.*

As 2 of the females from this family did not have any litters the first time they were put to breed, a second attempt was made after they had been 30 weeks on diet. Control femal No. 241 was allowed to have a second litter instead of being killed so that her young might act as a control to those of 245 and 246. After 33 weeks on diet No. 245 had a litter of 4, one born alive and 3 dead. At the *post mortem* of this female another fetus was found dead in the uterus. No. 246 also had a litter at the same time, but only one head was found as evidence that the litter had arrived. The second litter of the control was normal. The single live young of 245 was killed shortly after birth; the incisor teeth were found to be already abnormal in shape, especially towards the root, and the roots did not extend as far back beneath the molars as in the normal control at the same age. It was found impossible to make any satisfactory preparations from the dead head of 246's young.

When the lower jaws of these 6 mother rats were examined histologically, it was found that the incisor teeth of 243–247 had been af-

fected to some extent by the vitamin A deficiency; in particular the dentin of the labial side near the root was uneven and the outline of the teeth was broader and more squat than that of the control mothers.

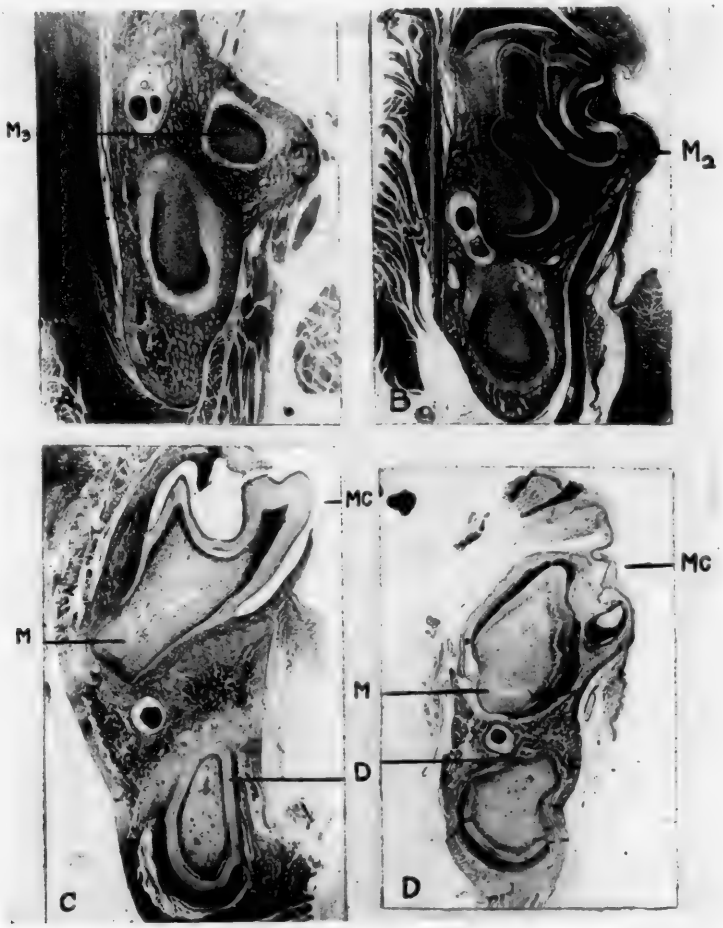

FIG. 7, A and B. T.S. through incisor root in 15 day old rats. × 40. A. Young of control rat 241 (incisor root extends back below third molar region). B. Young of vitamin A deficient rat 243 (incisor root only extends below second molar).

C and D. T.S. through first lower molar and incisor in 17 day old rats. × 40. C. Young of control rat 241. D. Young of vitamin A deficient rat 243.

More evidence of the effect of the A deficiency on their tissues was seen by examining the gingival and subgingival epithelium of the first lower molars. *Figs. 8A and B* are photomicrographs of this region in 2 mother rats. In the control (241) the subgingival epithelium is thin and ends at the cementoenamel junction (*fig. 8A*). In the A deficient mothers the subgingival epithelium showed various stages of abnormality. It is most abnormal in 243 (*fig. 8B*) which shows all the effects of vitamin A deficiency as described for these tissues by King (1940).

Conclusions from Series 4. The state of the teeth in the young from these litters was worse at a much earlier date than in any of the preced-

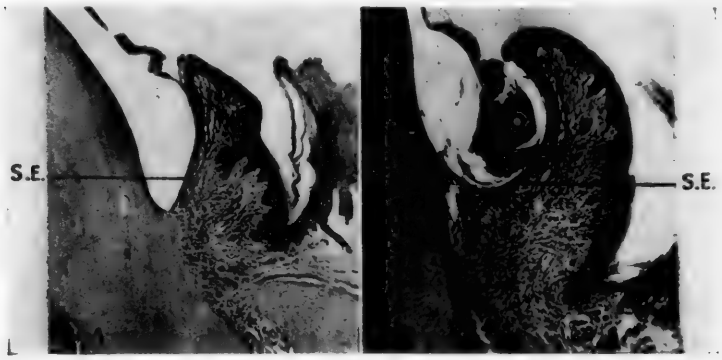

FIG. 8. T.S. of gingiva with subgingival epithelium from 1st lower molar of mother rats 241 and 243 after 28 weeks on diet. × 90. A. Control rat 241. B. Vitamin A deficient rat 243.

ing litters. The mothers were also very definitely affected, the gingival tissues in some cases showing advanced periodontal abnormalities; the mothers, however, were never as badly affected as their offspring.

Series 5. Females littering when 34 weeks old after 31 weeks on diet

Three female rats Nos. 271, 272, 273 were placed on diet at the age 23 days, No. 271 being a control animal. After 31 weeks on diet they were put to breed, with the result that one normal control litter was obtained, but both the A deficient mothers produced dead litters and additional dead fetuses were found at a *post mortem* examination of 272 and 273.

Series 6. Females littering when 37 weeks old after 34 weeks on diet

Six female rats from 2 litters were placed on diet at the age of 23 days. Nos. 261–263 were from one litter, and 264–266 from the other; 261 and 264 were made control animals. After 31 weeks on diet an attempt was made to get these rats to breed. Both the control rats produced normal litters after 34 weeks on diet, but only one vitamin A deficient rat became pregnant; her litter was mostly born dead, but 3 full term fetuses were removed from the uterus apparently alive. On sectioning the lower jaw of one of these the shape of the incisor tooth was found to be already abnormal.

The remaining 3 vitamin A deficient rats never showed any sign of pregnancy. Two became ill and were killed after 38 weeks on diet, while the third died after 39 weeks on diet. It seems probable that these rats were sterile as a result of continued vitamin A deficiency and in spite of adequate supplies of vitamin E from the wheat germ oil. Parks and Drummond (1926) attributed the sterility of female rats on an A deficient diet to physiological debility and failure to copulate. Tansley (1936) found that vitamin A deficient female rats had difficulty in casting their litters as a result of which nearly all the young were born dead. Newton (1938) using the same diet as Tansley, found that uncompleted pregnancy was more frequent than difficult labor; the addition of daily doses of wheat germ oil in his experiments made no difference to the pregnancies.

When the lower jaws of rats 262, 263, 265 and 266 were sectioned the molar gingival tissues and the incisors were found to be abnormal as compared with the controls.

Conclusions from Series 5 and 6. This and the foregoing experiment show that after a period of more than 30 weeks on this vitamin A deficient diet the rats are unable to produce live litters.

Series 7. Diet modified by substituting tocopherol acetate for wheat germ oil as a source of vitamin E

It has been realized that the wheat-germ oil used in the diet as a source of vitamin E was liable to contain a certain amount of vitamin A. During the course of the investigation synthetic vitamin E became available for the first time and it was decided to give this in the form

of tocopherol acetate[2] in place of the wheat-germ oil. The rats were given 1 mg. dissolved in olive oil, twice a week by placing the drops of oil in the rat's mouth.

In all 21 rats from 5 litters were used, 10 being controls and 11 vitamin A deficient. Unfortunately the A deficient females became adversely affected by the diet in an even shorter time than was anticipated, in some cases before experimental breeding was begun. This was probably because the diet was now without the small amount of vitamin A frequently present in the wheat-germ oil. Two virgin females became moribund after 17 weeks on diet and were killed along with two controls. The remaining 9 A deficient females were put to breed after shorter periods, i.e. 10–11 weeks (2 rats), 12–13 weeks (3 rats) and 15–15.5 weeks (4 rats).

The only live young obtained from an A deficient mother was one litter born when the mother was 14 weeks on diet. The results from this litter are described below. Only 2 other vitamin A deficient females became pregnant. One of these littered after being 18 weeks on diet, but the litter was eaten before it could be examined. The other female was killed after 18 weeks on diet because it was ill, and at the *post mortem* it was found to be pregnant. The remaining 6 females all failed to show any trace of pregnancy though litters were obtained from 7 out of 8 control animals.

The vitamin A deficient litter was obtained from rat 313 after she had been 14 weeks on diet. Rat No. 311 produced a control litter at the same time. The young of the former were very weakly and only survived to the age of 4 days, but material was examined at 1, 2 and 4 days. These young showed great abnormalities in their teeth as compared with the controls. In *fig. 9*, two day old young from the control and the vitamin A deficient mother are shown for a comparison of their incisor teeth. The sections pass through the lower incisor at a point where the teeth are at the same stage of development, but the sections are not at the same level in both jaws because the vitamin A deficient young is less developed and very much smaller in size, as a result of this the incisor of the A deficient young of rat 313 has a shorter root and any comparable point as far as development is concerned will be found in a more anterior position than that of the control young from rat

[2] Kindly supplied by Messrs. Roche Products Ltd.

311. *Fig. 9A* shows the abnormal shape of the incisor and the difference in the appearance of the bone of the lower jaw.

The first and second molars of the young from rat 313 were also noted to be abnormal. The dentin and enamel was laid down in a wavy line so that the amelo-dentinal junction had a corrugated outline in places.

Conclusions from Series 7. The use of tocopherol acetate in the diet in the place of the wheat-germ oil resulted in the vitamin A deficient females being adversely affected by the diet in a much shorter

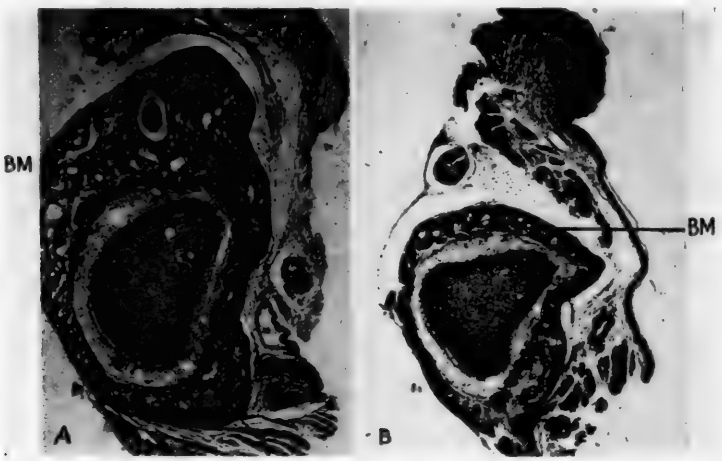

FIG. 9. T.S. Lower incisor root in 2 day old rats. A. Young of control rat 311. B. Young of vitamin A deficient rat 313.

time. The rats appeared to be sterile after about 15 weeks of diet and deaths occurred after 17 to 20 weeks of diet. The family obtained from one female after 14 weeks of diet would seem to be intermediate in its stage of A deficiency between those of rats 243, 244 and 262–265.

SUMMARY

1. Thirty female rats were fed from the age of 3 or 4 weeks on a diet deficient in vitamin A for several different periods up to 34 weeks before experimental breeding was begun. Many of these females produced young. Others (19 in all) taken from the same litters were

reared on the same diet plus a daily dose of mammalian liver oil as a source of vitamin A. These were allowed to breed at the same time as their vitamin A deficient litter-mates.

2. Females on the vitamin A deficient diet for 12 to 13 weeks before casting their litters produced young with normal dental tissues, in spite of the fact that the mothers' store of vitamin A in the liver was on the point of exhaustion, and the young, with one exception, had no store of vitamin A.

3. Longer periods on diet such as 15 to 19 weeks before the litters were born, resulted in the dental tissues of the mothers (incisor roots and molar gingivae) showing slight changes due to the vitamin deficiency. In their young much more marked effects were noted from the age of one week onwards. The young remained alive for 16 to 18 weeks.

4. After the mothers had been 24 to 25 weeks on diet prior to the birth of their litters then their teeth became more affected by the vitamin A deficiency. Three weeks after the birth of their litters the molar gingival tissue particularly, showed pathological changes. The dental tissues of the babies of these mothers showed gross abnormalities from the age of a few days; their incisor teeth were very abnormal in shape, the teeth were retarded in development, and although the molars appeared to be less affected than the incisors, they too were misshapen particularly as regards their roots. No young lived longer than 10.5 weeks and most died much earlier.

5. After about 30 weeks on diet, practically all the young were stillborn, and dead fetuses were found unexpelled from the uteri. Some females 34 weeks on diet apparently never became pregnant in spite of receiving adequate supplies of vitamin E. Fairly severe signs of vitamin A deficiency were noted in the dental tissues of these animals.

6. 21 rats were given synthetic tocopherol acetate as a source of vitamin E instead of wheat germ oil. Those receiving vitamin A (10) developed and reproduced normally, but the others (11) were adversely affected by A deficiency in about half the time in which those receiving the wheat germ oil showed similar reactions. It is evident that a very small intake of vitamin A, as may be found as an impurity in the wheat germ oil, may greatly delay the onset of gross A deficiency in females.

7. In all experiments where abnormalities occurred, these were more pronounced in the young than in their mothers.

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